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Review

Biological Hazards in Fish Production: Legionella in Aquaculture and Fisheries: A Potential Occupational Exposure?

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Abstract

The increasing global demand for fish and fishery products has established fisheries and aquaculture as essential sectors for food security and economic development. However, the intensification of these activities may also increase exposure to biological hazards inherent to aquatic environments and production systems, raising concerns for food safety, occupational and public health. Among these hazards, pathogenic bacteria, associated with water, sediments, and aquatic organisms, are of particular concern. Environmental changes, including climate change and the expansion of aquaculture practices, may further promote the emergence and dissemination of these microorganisms. Within this context, *Legionella* spp. is recognized as a relevant waterborne pathogen due to its widespread occurrence in natural and artificial aquatic environments and its capacity to cause human disease, primarily through the inhalation of contaminated aerosols. Consequently, workers involved in aquaculture, fisheries, and fish-processing industries may represent a population potentially exposed to this microorganism. Although legionellosis is not considered a zoonosis associated with fish consumption, and no occupational cases linked to these sectors have been reported to date, the persistence of *Legionella* in aquatic environments highlights a plausible but insufficiently investigated exposure pathway. This narrative review synthesizes information from SciELO, Scopus, Google Scholar, and other relevant bibliographic sources on the biological and clinical characteristics of *Legionella*, available detection methods and prevention strategies. The aim is to examine the potential for occupational exposure within fish production systems and discuss its possible implications for worker health. By identifying hypothetical exposure scenarios and current knowledge gaps, this review seeks to support future research and contribute to the prevention and assessment of biological risks in the fisheries and aquaculture sectors, without assuming the existence of a demonstrated occupational hazard or an emerging public health problem.

Keywords: *Legionella*; waterborne pathogens; aerosol exposure; potential health risk

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1. Introduction

Food safety and quality during production are matters of public health importance [1]. The quality of fish and fishery products is determined by their freshness and by the presence of physical, chemical, or biological hazards that contribute to spoilage and health risks [1–3].

A wide variety of biological hazards, including bacteria, viruses, fungi, and protozoa, can inhabit and persist in diverse natural environments such as soil, air, water, plants, animals, and humans. These hazards may be transferred to food at various stages of the supply chain, leading to product spoilage and illnesses [2,4].

Zoonoses are diseases or infections transmitted from vertebrate animals to humans [5–7]. They may be caused by bacterial, viral, parasitic, or fungal agents. Multiple factors contribute to their emergence and dissemination, including globalization, international trade, urbanization, deforestation, agriculture, food handling, changes in aquatic ecosystems, human–animal coexistence, intensive exploitation of domestic or farmed animals (including aquaculture), wildlife exploitation and trade, environmental modification, land-use changes, and climate change in both urban and rural contexts [7–11]. Transmission routes include ingestion of contaminated food, penetration through broken or abraded skin, bites, vectors (e.g., insects), inhalation of respiratory particles, contact with skin or mucous membranes, direct contact with diseased organisms, and exposure to contaminated water or environments [6,8,12,13]. It is estimated that approximately 60% of human infections are of animal origin [8,14], with most zoonoses arising through food systems encompassing production, processing, distribution, trade, and consumption [14].

Zoonoses associated with aquatic organisms have been reported to cause significant economic problems in aquaculture and fishing industries while posing risks to human health [12,13]. The growing global population and increased consumption of aquatic products have driven demand and trade of fishery and aquaculture products, which may heighten the risk of environmental contamination and the transmission of diseases from aquatic environments and organisms to humans [12].

Bacteria constitute the primary zoonotic agents associated with fish. Most are Gram-negative such as *Vibrio* sp., *Aeromonas* sp., *Pseudomonas* sp., *Yersinia* sp., *Mycobacterium* sp., *E. coli*, *Shigella* sp., *Klebsiella* sp., *Salmonella* sp., *Edwardsiella* sp., *Campylobacter* sp., and *Plesiomonas* sp., while others are Gram-positive (*S. agalactiae*, *S. iniae*, *E. rhusiopathiae*, *Staphylococcus* sp., *L. garvieae*, *Nocardia* spp., *Listeria* sp., and *Clostridium* sp.) [5,12,13].

Fish may acquire these pathogens either as part of the indigenous microbiota or through contamination of the aquatic environments resulting from agricultural activities, human and animal waste, household refuse, or wildlife [5]. Transmission to humans typically occurs via consumption of raw or undercooked fish, exposure to water contaminated with fish feces or mucus, or contact with the infectious agent through open wounds or abrasions [5,12,13]. Occupational and recreational exposure to aquatic environments also represents a risk, particularly for fish farmers, fishermen, traders and food handlers [10,12].

It has been estimated that approximately 1407 species of human pathogens have been identified, of which nearly 58% are zoonotic in origin [15]. Similarly, the World Organization for Animal Health (WOAH) reports that approximately 60% of infectious diseases affecting humans originate from domestic or wild animals [16]. Natural and artificial aquatic environments can act as reservoirs for a wide range of biological hazards, including opportunistic pathogenic bacteria such as *L. pneumophila*. The public health significance of these reservoirs depends on environmental conditions that promote bacterial survival, persistence, and proliferation, as well as on the presence of effective routes of human exposure, particularly through the inhalation of contaminated aerosols [4,5,12,17,18].

Among aquatic bacterial pathogens, *Legionella* spp. are of particular public health concern because of their ability to colonize both natural and artificial aquatic systems and

cause infection primarily through aerosol transmission. Consequently, workers involved in aquaculture, fishing, and fish-processing industries may represent a potentially exposed occupational group. Although legionellosis is not considered a fish-borne zoonosis and no cases of occupational infection associated with these sectors have been reported to date, the documented persistence of *Legionella* spp. in aquatic environments suggests a plausible, yet largely unexplored, scenario of occupational exposure through aerosol inhalation. This consideration highlights the importance of microbiological surveillance, risk assessment, and preventive measures aimed at safeguarding occupational and public health.

Accordingly, this narrative review analyzes information from SciELO, Scopus, Google Scholar, and other databases to provide an overview regarding biological hazards associated with fish production systems, including aquaculture, capture fisheries and fish-processing activities, with particular emphasis on *Legionella* spp. The review summarizes the biological characteristics of these bacteria, their ecology in aquatic environments, transmission pathways, clinical relevance, and potential implications for occupational health. Although no occupational cases have been documented in these sectors, the available evidence supports the recognition of *Legionella* spp. as a biological hazard and suggests hypothetical occupational exposure scenarios associated with aerosol-generating activities. By identifying existing knowledge gaps and highlighting potential exposure pathways, this review aims to encourage future research and support preventive approaches for protecting worker health throughout the fish production chain.

The information presented in this narrative review was identified, compiled and critically analyzed using multiple databases (SciELO, Scopus, PubMed, Google Scholar, among others), complemented by relevant institutional reports and the gray literature. Publications written in English and Spanish were considered, with no restriction on publication date. The literature search was conducted using Boolean operators and combinations of keywords related to fish production systems, occupational health, biological hazards, food safety, and *Legionella* spp. Representative search terms included fish, occupational exposure, risk, zoonosis, biological hazards, aquaculture, fishing, fish processing, aerosols, pathogens, *Legionella*, waterborne pathogens, foodborne diseases, control systems, public health, "Legionellosis", "Microbiological hazards in fish", "Foodborne disease", "Occupational exposure to *Legionella*", "*Legionella* and health", "Occupational health", "Fish-borne zoonosis", "Zoonosis in aquaculture and fishing", "Aquaculture and fishing" and "Fish food safety". Titles and abstracts were initially screened to assess their relevance to the objectives of the review. Full-text articles were subsequently evaluated when available and considered pertinent. Studies addressing biological hazards associated with fish production systems, risky practices, disease prevention, and occupational safety regarding pathogen exposure were included. Studies focused exclusively on the clinical studies of legionellosis without relevance to occupational or environmental exposure, as well as occupational health studies unrelated to *Legionella* spp. or fish production activities, were excluded from the analysis.

2. Fish

Fish is defined as food derived from oceanic or inland waters intended for human or animal consumption. The term encompasses fish, crustaceans, mollusks, algae, and other aquatic organisms [1]. Fish is considered a highly nutritious component of the human diet, serving as a primary source of easily digestible, high-biological-value protein due to its essential amino acid profile. It is also valued for its content of unsaturated fatty acids, fat-soluble vitamins (A and D), water-soluble B vitamins, and minerals such as calcium, iodine, zinc, phosphorus, and selenium [1,3,19]. Furthermore, the nutritional properties of fish have been associated with beneficial effects against cardiovascular diseases, cancer, and neurodegenerative conditions such as Alzheimer's disease [19].

Aquatic foods intended for human consumption are obtained primarily through fishing and aquaculture activities. These sectors play a fundamental role in global food security, nutrition, and economic development. According to recent reports, global aquatic animal production from fisheries and aquaculture reached 194.6 million tons (live weight) in 2024, of which nearly 89% was destined for direct human consumption, corresponding to a per capita consumption of 21.3 kg. Additionally, the total export value of aquatic products from fisheries and aquaculture reached USD 184 billion, while 65 million individuals were employed across these primary sectors, underscoring their contribution to nutrition, economic development, and social well-being [20].

Globally, aquatic foods of animal origin intended for human consumption are distributed in various forms: live, fresh or chilled, frozen, cured (dried, salted, fermented, smoked), or prepared and preserved [20,21]. Distribution varies by region: in high-income countries, products are predominantly frozen, preserved, or cured products, with an expanding range of easy-to-prepare and ready-to-eat options. In contrast, in many low- and middle-income countries, a significant proportion of production is distributed as live, fresh, or chilled products, which generally exhibit a shorter shelf life due to limited cold chain infrastructure, consumer preferences, and the predominance of small-scale supply systems [20]. In addition to the susceptibility of fish to spoilage, which stems from its intrinsic properties [22].

3. Foodborne Zoonotic Diseases

Foodborne zoonotic diseases continue to be a major public health challenge worldwide, particularly in low- and middle-income countries, due to their associated morbidity, mortality, and socioeconomic impact, including healthcare costs and productivity losses [5,23–25]. These illnesses may result from the consumption of food or water contaminated with pathogenic microorganisms, including bacteria, viruses, and parasites or from the ingestion of microbial toxins [23–25]. Clinical manifestations range from mild gastrointestinal symptoms, such as nausea, vomiting, abdominal pain, and diarrhea, with or without fever, to severe outcomes including septicemia, meningitis, pregnancy-related complications, and post-infectious disorders such as Guillain–Barré syndrome [26]. The burden of foodborne diseases is further influenced by factors including population growth, increasing food demand, globalization of food supply chains, environmental contamination, and climate change [27]. Vulnerable populations, particularly children under five years of age, older adults, pregnant women, and immunocompromised individuals, are at increased risk of severe disease and adverse health outcomes following exposure to foodborne pathogens [10].

Foodborne bacterial diseases are generally classified into three categories according to their pathogenesis. The first category comprises foodborne infections, which occur following the ingestion of viable pathogenic microorganisms that subsequently colonize, invade, or multiply within the host. Representative pathogens include *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Campylobacter* spp., and specific pathogenic strains of *E. coli*. The second category includes foodborne intoxications, which result from the ingestion of preformed toxins in food consumption, such as those associated with *Bacillus cereus* (emetic type), *Staphylococcus aureus*, and *Clostridium botulinum*. The third category comprises foodborne toxicoinfections, in which ingested microorganisms proliferate in the intestinal tract and subsequently produce toxins in situ, as observed with *Clostridium perfringens* and diarrheagenic strains of *Bacillus cereus* [23,25,28,29].

Fish and fishery products have been linked in foodborne illness outbreaks caused by a diverse range of microbiological hazards. Among the bacterial pathogens most frequently associated with these products are *Salmonella* spp., *Listeria monocytogenes*, *S. aureus*, and *Vibrio* spp., although the significance of specific hazards varies according to product type,

geographical origin, production practices, and storage environments [30–32]. Factors that contribute to contamination and subsequent illness transmission include inadequate hygienic practices during production and handling, insufficient temperature control during storage and distribution, cross-contamination, and the consumption of raw or undercooked seafood products [5,10,23,31,32].

It is important to distinguish these conventional foodborne hazards from microbiological hazards associated with aquatic environments and water systems. Although *Legionella* may be present in natural aquatic environments and in water distribution systems linked to aquaculture facilities or seafood-processing plants, their occurrence in these environments does not imply that fish consumption represents a route of transmission for legionellosis. Unlike classical foodborne pathogens, *Legionella* spp. are primarily transmitted through the inhalation of aerosols containing contaminated water droplets. Currently, fish consumption has not been recognized as an established route of transmission, and there is no robust epidemiological evidence identifying the ingestion of fish or fish products to the occurrence of legionellosis.

Consequently, the relevance of *Legionella* spp. in aquaculture and fisheries should focus on their presence in aquatic systems, the conditions that favor their proliferation, and potential routes of environmental or occupational exposure, particularly the generation of aerosols, all of which will be addressed in the following sections.

4. Fish Microbiology

The microbial population of fish is influenced by habitat, environmental conditions, and processing surfaces [3,22,33,34]. Prevalence depends on water microbiota, handling practices, and hygienic conditions [3,22]. While the muscle tissue of healthy live fish is sterile, microbial communities are commonly associated with the external and internal surfaces, particularly the skin, gills, and digestive tract [4]. The microbial load on fish skin typically ranges from 10^2 to 10^7 CFU/cm^2, whereas bacterial populations in the gills and intestinal contents generally range from 10^3–10^9 CFU/g [34,35].

Bacteria associated with fish can be classified into two categories: (a) The first is autochthonous bacteria, which are indigenous to aquatic environments and constitute part of the natural microbial community of the habitat in which fish reside. Representative microorganisms include *C. botulinum*, *Vibrio* spp., *Plesiomonas* spp., *Mycobacterium* spp., *Edwardsiella tarda*, and *Aeromonas* spp. These microorganisms commonly colonize the skin, gills and gastrointestinal tract of fish, and their prevalence is influenced by environmental factors, particularly water temperature. (b) The second category is non-autochthonous bacteria, such as *E. coli*, *Shigella* spp., *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp., which originate from human or animal reservoirs. The presence of these microorganisms in fish and aquatic environments is generally associated with fecal contamination and inadequate sanitary conditions [33,36,37].

Fish may harbor a variety of bacterial species of veterinary and public health significance, including *Mycobacterium* spp., *Streptococcus* spp., *Vibrio* spp., *Aeromonas* spp., *Edwardsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Listeria* spp., and *Clostridium* spp., among others [4,12,38]. However, the zoonotic potential and public health relevance of these microorganisms vary considerably, and not all bacteria associated with fish are zoonotic in the strict sense. Some bacterial species may form part of the microbiota of apparently healthy fish without causing disease under normal conditions, yet they can behave as opportunistic pathogens when environmental stressors, compromised host immunity, high stocking densities, or poor water quality disrupt host–pathogen-environment interactions [39,40].

In addition to these microorganisms, bacterial genera of public health concern, such as *Legionella*, have been detected in aquatic environments associated with fish production sys-

tems. Their presence is often linked to aquatic environments rather than the fish microbiota itself [41,42]. For example, Smith et al. [43] reported the presence of *L. pneumophila*, *V. vulnificus*, *V. cholerae* and *A. hydrophila* in ornamental fish aquaria, highlighting these environments as potential reservoirs of microorganisms relevant to animal and human health.

As aquaculture and fishery production expands, the likelihood of zoonotic infections through handling or ingestion of fish products increases [36]. Fish and fish products have been implicated in zoonotic diseases primarily involving bacteria (Table 1), particularly when production, handling, processing, or consumption occurs under poor hygienic conditions [4,10,33,36]. Infections caused by fish-borne pathogens are influenced by seasonality, dietary habits, immune status, and exposure to aquatic environments [33]. In aquaculture, zoonotic transmission is associated with factors such as nutrient levels, water quality, and high stocking densities, which promote high microbial proliferation on fish surfaces [36].

Table 1. Major bacterial hazards of public health concern associated with fish and fishery.

Bacterium	Type	Conditions for Causing Disease	Source
Aeromonas hydrophila	Opportunistic pathogen/potential zoonotic and foodborne pathogen	Direct contact through puncture or wounds. Product consumption.	[11,12,36,44–46]
Plesiomonas shigelloides	Opportunistic Pathogen/enteric pathogen	Product consumption	[37,38,45]
V. vulnificus *V. damsela* *V. parahaemolyticus*	Foodborne pathogen/opportunistic pathogen Fish pathogen/zoonotic and opportunistic potential Foodborne pathogen	Direct contact through puncture or wounds. Product consumption.	[5,12,25,36,45–47]
Salmonella spp.	Foodborne pathogen/Enteric pathogen	Product consumption	[5,25,45,46]
Shigella sp.	Foodborne pathogen/Enteric pathogen	Product consumption	[5,33,37]
Streptococcus iniae *Streptococcus agalactiae*	Fish pathogen with zoonotic potential	Direct contact through puncture or wounds. Product consumption.	[5,11,36,45,48,49]
Clostridium perfringens *Clostridium botulinum*	Commensal and opportunistic pathogen/toxigenic Toxigenic pathogen	Product consumption	[5,25,38,45]
Edwardsiella tarda	Opportunistic Pathogen/Enteric pathogen/zoonotic potential	Direct contact through puncture or wounds. Product consumption.	[5,11,37,45,48,49]
Listeria monocytogenes	Foodborne pathogen and opportunistic pathogen in certain contexts	Product consumption	[5,25,45]
Mycobacterium marinum	Opportunistic pathogen	Direct contact with fish through puncture or wounds during handling.	[5,36,45,48,49]
E. rhusiopathiae	Opportunistic and zoonotic pathogen	Direct contact with skin wounds during handling and fish processing.	[5,48,50]
Lactococcus garvieae	Fish pathogen and zoonotic potential	Direct contact with skin wounds during fish handling, processing and consumption.	[5,45,51]
Nocardia spp.	Opportunistic pathogen	Direct contact with fish during handling and the presence of wounds.	[5]
Photobacterium damselae	Aquatic animal pathogen/zoonotic potential/Opportunistic pathogen	Direct contact with salty water, through puncture or wounds, fish handling and aquaculture tools.	[46,52]

5. *Legionella*

Bacteria belonging to the genus *Legionella* (family *Legionellaceae*) are Gram-negative, non-spore-forming, non-encapsulated, motile, and strictly aerobic coccobacilli that are urease-negative [18,53,54]. These environmental microorganisms are widely distributed by both natural and aquatic ecosystems. They have been isolated from freshwater habitats, such as lakes, rivers, ponds, and streams, as well as from moist soils, sludges, thermal waters, composted material, and seawater. *Legionella* spp. are capable of surviving within a temperature range of approximately 20–50 °C and are generally inactivated at temperatures exceeding 70 °C. Their environmental persistence is enhanced by their ability to parasitize free-living aquatic and soil protozoa and to colonize biofilms within water systems. Intracellular survival and replication within protozoan hosts occur optimally at approximately 35 °C [18,53,55–60]. In addition to their broad ecological distribution, *Legionella* spp. exhibit considerable environmental adaptability and have been recovered from habitats with pH values ranging from 2.7 to 8.3 [56]. These bacteria readily colonize a variety of artificial water systems, including supply and distribution networks, wastewater treatment plants, cooling towers, evaporative condensers, ornamental fountains, spas, swimming pools, potting substrates, compost, and other facilities requiring water circulation [18,53,56–60].

L. pneumophila is a facultative bacillus measuring 0.3 to 0.9 µm in width and 1.5 to 15 µm in length. It requires amino acids, iron and cysteine for growth, and tests positive for catalase, oxidase, and gelatinase [18,61]. The bacterium survives within a temperature range of 25–45 °C, with optimal replication occurring between 32 and 42 °C [56,62]. In aquatic ecosystems, *L. pneumophila* exists either as free-living planktonic forms or as intracellular parasites of protozoa such as *Tetrahymena* and amoebae (*Acanthamoeba*, *Hartmannella*, *Naegleria*, *Vahlkamphia*, *Echinamoeba*). These protozoan hosts protect the bacterium from adverse conditions (e.g., acids, biocides, antibiotics, thermal and osmotic stress) and act as natural amplifiers within biofilms [18,55,57,59,62].

In engineered aquatic systems, *Legionella* spp. are predominantly associated with complex multispecies biofilms, which can complicate their detection, characterization, and control. Experimental biofilm models have demonstrated that the replication and persistence of *L. pneumophila* within these microbial communities are strongly influenced by the presence of protozoan hosts [55].

Furthermore, several bacterial species commonly found in aquatic biofilms, such as *Pseudomonas* sp., *Klebsiella pneumoniae*, *Flavobacterium* sp., and *Empedobacter breve,* may facilitate the presence and persistence of *L. pneumophila* in biofilms, posing a public health threat [54,57].

Among the currently described *Legionella* species, those of greatest clinical relevance include *L. pneumophila*, *Legionella bozemanae*, *L. micdadei*, and *L. longbeachae*, all of which have been implicated in human legionellosis. Of these, *L. pneumophila* is responsible for approximately 90% of reported cases worldwide. Within this species, serogroup 1 is the most frequently associated with human infection, despite the existence of at least 15 recognized serogroups [18,55,59,61,63,64]. Serogroup diversity reflects immunological variability within the species; surface-associated structures, particularly lipopolysaccharides and outer membrane proteins, serve as the principal determinants of serogroup specificity [65].

Legionellosis is predominantly associated with artificial aquatic environments that maintain water at elevated temperatures. Numerous outbreaks have been linked to cooling towers, evaporative condensers, and air-conditioning systems, all of which can generate respirable contaminated aerosols that may subsequently be inhaled by exposed people. Consequently, the widespread use and proliferation of such artificial systems have been recognized as a major factor contributing to increased opportunities for human exposure to *Legionella* in recent years [55].

Globally, legionellosis is estimated to cause between 8000 and 18,000 cases annually; however, the true incidence is likely higher due to underreporting, inconsistent sampling, limited diagnostic capacity in hospitals, and restricted surveillance programs, particularly in low-income countries [59]. The disease is notable in several countries and regions, including Canada, the United States, Japan, New Zealand, Singapore, Australia, and Europe [66]. Within the European Union and the European Economic Area, 11,298 cases were reported in 2019 compared to 6947 in 2015, underscoring a rising trend and highlighting the significant public health threat posed by the ubiquity of *Legionella* in aquatic systems [57]. In the United States, 184 outbreaks of waterborne disease associated with *Legionella* and biofilm presence were reported between 2015 and 2020, indicating a significant public health hazard [67]. Outbreak sources included building water systems, cooling towers, decorative water features, and other common water uses, often linked to deficiencies in water quality management, particularly in operating conditions and maintenance practices [66]. Legionellosis can occur in both hospital and community settings [53], manifesting either as outbreaks or isolated cases. Transmission results from the inhalation or aspiration of aerosols (airborne water droplets) generated by sprays, jets, or mists of contaminated water [57,58,60,68]. Once inhaled, *L. pneumophila* enters alveolar macrophages, replicates, and causes inflammation and tissue damage [59].

The pathogenicity of *Legionella* in humans is considered a consequence of its adaptation to intracellular survival within environmental protozoa [55,69]. Following the inhalation of contaminated aerosols, *L. pneumophila* is phagocytosed by alveolar macrophages, which constitute the first line of cellular defense in the lower respiratory tract. However, a characteristic of *L. pneumophila* pathogenesis is its ability to evade intracellular killing and establish a replicative niche within these host cells [69–71]. To achieve this, the bacterium remodels the phagosome into a specialized intracellular compartment known as the *Legionella*-containing vacuole (LCV), which supports bacterial survival and replication [69]. The formation and maintenance of the LCV are mediated by the Dot/Icm type IV secretion system (T4SS), a major virulence determinant that translocates hundreds of effector proteins into the host cell cytoplasm [55,69,72]. These proteins alter vesicular trafficking, the cellular response, and other cellular pathways, thereby enabling intracellular survival [55].

Intracellular replication ultimately results in host–cell lysis and the release of large numbers of bacteria into the surrounding tissue, where they subsequently infect neighboring cells. This process induces the production of chemotactic factors, leading to the recruitment of monocytes, macrophages, and polymorphonuclear neutrophils to the site of infection. The resulting inflammatory response is accompanied by increased vascular permeability, alveolar edema, serum transudation, and fibrin deposition within the alveolar spaces, culminating in pulmonary tissue damage and impaired respiratory function. Clinically, these pathological events manifest as Legionnaires' disease, a potentially severe form of pneumonia associated with significant morbidity and mortality [59,65,70].

In addition to the Dot/Icm secretion system, *Legionella* possesses a diverse array of virulence factors that contribute to host colonization, intracellular infection and pathogenicity. These include surface structures such as lipopolysaccharides, flagella, pili, and outer membrane proteins, as well as a type II secretion system (T2SS) that secretes degradative enzymes. Other critical factors include the peptidyl-prolyl isomerase Mip (Macrophage Infectivity Potentiator) and iron acquisition mechanisms [55,69,71,73].

Person-to-person transmission of *Legionella* is extremely rare [74], with only one probable case documented to date [57]. The bacterium is not part of the normal human microbiota [66]. Legionellosis has an incubation period of 2–10 days and a mortality rate of 5–10%, resulting from progressive pneumonia complicated by respiratory failure, septic shock, or multi-organ dysfunction [60,75].

Clinically, legionellosis presents in two distinct forms: (1) legionnaires' disease (pneumonic form), characterized by fever, malaise, weakness, loss of appetite, lethargy, headache, cough, diarrhea, pleuritic pain, and myalgia, and (2) pontiac fever (non-pneumonic form), with symptoms such as fever, chills, headache, malaise, and muscle pain [18,60,76]. Populations at greatest risk include hospitalized patients, immunocompromised individuals, patients undergoing chemotherapy, adults over the age of 50, and those with chronic respiratory or renal diseases. Additional risk groups include diabetics, smokers, heavy drinkers, newborns delivered via water birth, and individuals living or working near water distribution systems, cooling towers, swimming pools, or other aquatic environments [18,60,62,77]. Occupationally exposed groups include maintenance workers, water service providers, cleaning and wastewater treatment personnel, and workers in industries utilizing industrial water sprayers [77]. The standard therapeutic approach for legionellosis involves macrolides or fluoroquinolones, which remain the first-line agents for effective management [54].

5.1. Legionella in Fisheries, Aquaculture, and Human Health

Legionella spp. are ubiquitous in both natural (lakes, rivers, and streams) and artificial (distribution and supply systems) water systems worldwide [54,78]. Water is considered the primary reservoir of these microorganisms, while their persistence in artificial systems is largely facilitated by their association with protozoa and biofilms [54,59,78]. In aquaculture environments, diverse bacterial communities have been reported, including pathogenic and opportunistic genera such as *Legionella*, *Lactococcus*, *Aeromonas*, *Chryseobacterium*, *Pseudomonas*, *Mycobacterium*, *Escherichia* and *Shigella*, highlighting the potential relevance of these systems to animal and public health [45,79]. Evidence supporting the presence of *Legionella* in aquaculture environments remains limited but noteworthy. For example, Moosavian and Dashti [63] detected *L. pneumophila* in fishpond water samples using both culture- and PCR-based methods. Their study demonstrated greater detection rates using molecular techniques, with PCR exhibiting 100% sensitivity and 95% specificity. Similarly, Tabatabaei et al. [80] identified *L. pneumophila* in fishpond water through culture and PCR assays targeting the *icmO*, *sidA* and *lidA* genes. In that study, 35.7% of water samples tested positive for at least one of the target genes, whereas viable isolates were recovered from a smaller number of samples through culture methods. These findings suggest that aquaculture water systems may act as environmental reservoirs of *Legionella* and support the need for further investigation into their occurrence and persistence in such environments.

Despite these observations, evidence directly linking *Legionella* to aquaculture systems remains limited when compared with other aquatic bacterial pathogens. Furthermore, many studies report the presence of *Legionella* based on high-throughput sequencing approaches, particularly 16S rRNA gene analyses, rather than culture or targeted molecular detection methods, which may limit conclusions regarding bacterial viability and epidemiological significance (Table 2). To date, there are no published occupational case reports, epidemiological studies, or quantitative exposure assessments specifically addressing *Legionella* exposure among aquaculture, fisheries, or fish-processing workers. The detection of a microorganism in an environment does not, by itself, demonstrate the existence of an effective occupational biological risk. Comprehensive risk assessments must also consider microbial viability, virulence characteristics, routes of transmission, exposure intensity and frequency, host susceptibility, and the specific activities performed within the workplace [81].

Legionellosis is recognized as an occupational disease associated with exposure to contaminated water systems and aerosols generated in occupational and environmental exposures [57,78]. Documented occupationally exposed groups include personnel responsible for water supply systems, hot-water distribution networks, heating, ventilation and

air-conditioning (HVAC) systems, thermal-water facilities, rehabilitation and recreational centers, aerosol therapy equipment, and wastewater treatment plants [57].

Table 2. Studies that reported on the presence of *Legionella* in aquaculture systems.

Location	System/Organism	Sample Type	Source
Canada	Recirculating Aquaculture Systems (RAS)	RAS compartments, especially biofilters and water.	[82]
Not specified	Fish kept in aquarium conditions	Gastrointestinal tract, particularly the foregut; also compared with tank water.	[41]
Studies from different countries	Marine and freshwater recirculating aquaculture systems (RAS)	Primarily culture water and biofilters.	[83]
Spain	Marine recirculating aquaculture systems (RAS) and aquaponic system; fish (*Salicornia patula*, *Mugil cephalus*)	Fish intestine, biofilters, biofilms, and water	[84]
China	Two Recirculating Aquaculture Systems (RAS): one in the setup phase and the other stocked with fish.	Water and bacteria adhering to K3 biomedia/biofilters	[85]
United States	Aquaculture/ornamental sector—marketing systems/*Carassius auratus* and *Gyrinocheilus aymonieri*	Fish-associated tank water	[43]
China	Aquaculture/eels	Aquaculture culture water, wastewater, effluents, raw water, sediments, and biofilms from various points on farms	[79]
Not specified	Recirculating Aquaculture Systems (RAS)/Aquaponics/Tilapia	Tank water, wastewater	[44]

Based on these observations, aquaculture, fishing, and fish-processing activities may represent environments in which *Legionella* can persist and where occupational exposure to contaminated aerosols could hypothetically occur. However, legionellosis is not considered a fish-borne zoonosis, and no confirmed cases have been reported among workers involved in aquaculture, commercial fisheries, or fish processing. Reported occupational cases involving maritime personnel have instead been linked to contaminated onboard water systems. For example, in Australia and Spain, separate cases of Legionnaires' disease and Pontiac fever have been documented among crew members of commercial vessels, with *Legionella* detected in showers, taps, and other components of shipboard water systems [86,87]. Nevertheless, such findings should not be directly extrapolated to aquaculture or fisheries operations because the environmental conditions and exposure scenarios differ substantially.

The absence of documented cases among aquaculture and fisheries workers should not be interpreted as evidence of the absence of risk. Rather, it may reflect underdiagnosis, underreporting, challenges in source attribution, limited occupational and environmental surveillance, and the multifactorial nature of exposure pathways [88]. These factors may contribute to an underestimation of the true extent of exposure in occupational settings associated with aquatic environments.

The presence of *Legionella* in aquatic environments underscores the need for enhanced microbiological surveillance and hazard identification, particularly where conventional systems are limited. Workers in aquaculture and fisheries may be exposed to aerosols during activities including water aeration, operation of diffusers and pumps, tank cleaning, equipment maintenance, fish harvesting, use of high-pressure washing systems, operation of ice-production facilities, and contact with hot-water systems or showers [35,78,89]. Because *Legionella* can exist as free-living bacteria, intracellular parasites of protozoa, or components of biofilm communities [18,53,56–60], these environments may provide suitable ecological niches for their persistence.

Several environmental factors relevant to aquaculture systems may contribute to the survival and proliferation of *Legionella*, including water temperatures between 25 and 45 °C, pH conditions within the bacterium's tolerance range (2–9.5), the accumulation of organic matter, the presence of biofilms, water stagnation, and the occurrence of protozoan hosts [43,56,62,78,90]. Aquaculture facilities operate under diverse environmental and management conditions, and water temperatures vary according to geographic location, production system design, species requirements, and thermal control practices [90]. Consequently, some production systems may provide environmental conditions conducive to *Legionella* persistence. Nevertheless, temperature alone is insufficient to predict the presence or proliferation of the bacterium, as additional ecological and microbiological factors influence its population dynamics. Therefore, confirmation of *Legionella* occurrence in aquaculture facilities requires targeted microbiological monitoring and environmental assessment. Ultimately, the significance of these bacteria for human health depends not only on their presence in aquatic environments but also on the generation and inhalation of contaminated aerosols, which constitute the primary route of transmission.

5.2. Isolation and Detection of Legionella

Culture remains the reference method for detecting *Legionella* in water samples. Selective GVPC media (cysteine, glycine, vancomycin, polymyxin B, and cycloheximide) and buffered charcoal yeast extract (BCYE) agar supplemented with L-cysteine, ferric pyrophosphate, or antibiotics [59,61,63,68,76,80,91] are commonly employed. Isolates are subsequently identified through biochemical tests such as Gram staining, oxidase, gelatinase, and hippurate hydrolysis [63,80]. Standardized protocols, including ISO 11731 [92], provide guidance for the isolation and quantification of *Legionella* from a wide range of environmental matrices, including drinking water, industrial water, wastewater, natural water sources, biofilms, and sediments. However, culture-based methods alone may underestimate the occurrence of *Legionella*, as not all species or physiological states are readily culturable under standard laboratory conditions. Consequently, complementary molecular approaches, such as PCR-based assays and other nucleic acid detection techniques, are frequently employed to improve detection sensitivity and specificity.

Given the limited availability of standardized methodologies specifically designed for aquaculture facilities, fisheries environments, and aquatic product processing systems, analytical procedures validated for other water matrices may serve as a useful reference framework for environmental monitoring. Nevertheless, the direct transfer of such methodologies to aquaculture-related settings should be approached with caution, as differences

in water quality characteristics, organic matter content, microbial communities, and operational conditions may influence analytical performance.

Although isolation and culture are commonly used, standardized processes featuring easily interpretable schemes [22,91] generally offer several advantages: 1. comparability across studies using standardized protocols; 2. quantification of viable cells; and 3. recovery of clinical or environmental isolates for epidemiological linkage between water sources and infection cases [93]. Despite these strengths, culturing presents limitations: prolonged incubation (10 to 14 days) for colony identification, with no single standard duration (though 10 days is common); interference from fast-growing biota that inhibit or hinder isolation; variability in colony identification; sample preparation inconsistencies; and inability to detect viable but non-culturable (VBNC) cells [68,93,94]. Experimental stages typically include sample concentration, decontamination (heat or acid treatment), selective media inoculation, incubation, enumeration, and confirmation and typing [18,63,80].

Another approach for the detection and quantification of *Legionella pneumophila* in water samples is the Most Probable Number (MPN) method [59,93]. This culture-based technique provides an alternative quantitative approach, particularly for samples with elevated levels of background microbiota. The method typically involves the pre-treatment of non-potable water samples, inoculation into selective liquid media, and incubation at 37–39 °C for approximately seven days. Enumeration is subsequently determined based on microbial growth indicators, such as turbidity or colorimetric changes, which are interpreted using standardized MPN reference tables. Compared with conventional plate-counting methods, the MPN approach offers operational simplicity, improved selectivity, and, in some cases, higher recovery rates of *Legionella* spp. in samples containing abundant non-*Legionella* microorganisms [94]. Consequently, the MPN method may be useful for estimating *Legionella* concentrations based on growth/no-growth responses and can be considered a semi-quantitative microbiological approach for specific environmental matrices. However, its performance in aquaculture, fisheries, and fish-processing environments has not been extensively investigated. Therefore, its applicability to these systems requires further validation, and it should not be regarded as a primary analytical method until its sensitivity, specificity, and reproducibility have been evaluated under matrix-specific conditions.

The identification of *Legionella* isolates from both clinical and environmental samples has been significantly enhanced by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) [76,91,95]. This rapid and cost-effective technology enables species-level identification through the analysis of characteristic protein profiles, primarily ribosomal proteins, generating highly reproducible spectral fingerprints. As a result, MALDI-TOF MS has emerged as a valuable tool that complements, and in some applications may replace, conventional biochemical and molecular identification methods [91]. Nevertheless, because the technique requires prior isolation and cultivation of the organism, its use remains dependent on successful culture recovery and therefore does not circumvent the limitations associated with culture-based detection methods.

Molecular techniques provide speed, specificity, and sensitivity, enabling detection of *Legionella* at very low concentrations, including VBNC cells, or intracellular forms within amoebae [68,93,94]. Conventional, real-time, and multiplex PCR methods targeted genes such as 5S, 16S, and *23S rRNA*, *mip* or *dotA* [59,75,76]. Additionally, standardized methods, including the Association Française de Normalisation (AFNOR) standard NF T90-471 and the International Organization for Standardization (ISO) standard ISO/TS 12869:2019, support quantitative PCR (qPCR) for detection and quantification in water samples [93]. Molecular methods complement conventional culture-based approaches and frequently enable the detection of *Legionella* spp. in samples that yield negative culture results. These techniques are particularly valuable for the rapid screening of large numbers of environ-

mental samples and for surveillance programs requiring high analytical throughput [96]. However, the performance of molecular assays may be affected by the presence of PCR inhibitors commonly encountered in environmental matrices, including humic substances, ferric ions, and other mineral compounds. Consequently, the implementation of efficient DNA extraction procedures, inhibitor removal strategies, and appropriate internal and external controls is essential to ensure analytical sensitivity, specificity, and reliability [96,97]. Figure 1 summarizes the principal methodological approaches currently available for the detection, identification, and quantification of *Legionella* spp. in aquatic environmental samples. The selection of an analytical method should be guided by the specific objectives of the investigation, including bacterial isolation, viability assessment, enumeration, species identification, presence/absence determination, or rapid screening requirements. Additional considerations include sample characteristics, the complexity of the environmental matrix, and the abundance of accompanying microbiota.

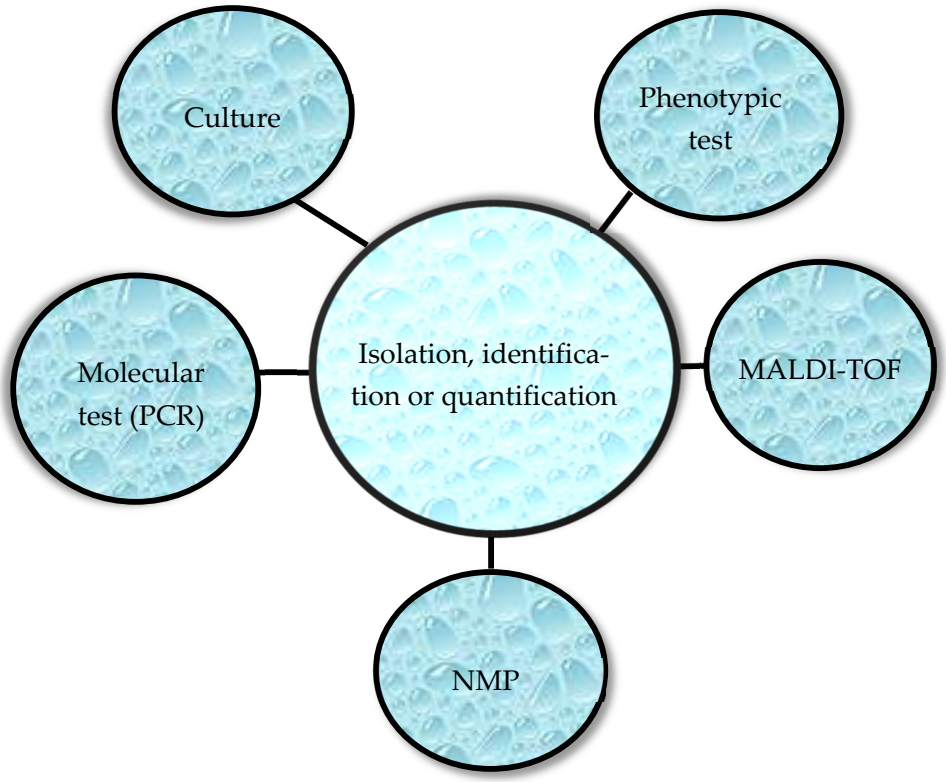

Figure 1. Profile of analytical methods for the study of samples from aquatic environments. Prepared by the authors.

I. Culture allows for the recovery of cultivable *Legionella* and enables further testing on isolates (such as species identification, serogrouping, and genomics).
II. PCR allows for the detection of genetic material even when culture fails to recover the microorganism; it serves as a rapid screening tool, useful for processing large numbers of samples and identifying priority ones.
III. MALDI-TOF complements culture by aiding in the identification of the obtained isolate.
IV. MPN should not be used as the primary method for *Legionella* analysis; it is difficult to use for direct counting, though it does allow for work with low concentrations.

5.3. Prevention and Control of Zoonoses

Currently, no vaccines are available for legionellosis as a public health preventive measure [60]. Therefore, understanding the ecological niche and habitat of *Legionella* is fun-

damental to infection prevention and control [18,76]. Prevention measures for the general population require coordinated action by health authorities, increased public awareness, and continuous monitoring of hygiene protocols. Regular microbiological inspections of water systems and chlorine treatments in public or community facilities are essential to reduce risks [58,94].

Individuals engaged in aquaculture, fishing, and fish-processing activities must be informed about zoonotic diseases and their prevention. Health authorities and occupational safety personnel should provide education on microorganisms associated with aquatic environments and the risks of consuming raw or undercooked fish [8,13,74]. Training should emphasize hygienic practices in veterinary medicine, occupational health, food hygiene, and personal hygiene [8]. Personnel working with fish should be adequately informed about zoonotic diseases, their routes of transmission, and the preventive measures available to reduce the risk of infection. Given that complete avoidance of contact with water, aquatic organisms, and potentially contaminated environments is impractical in most occupational settings, the implementation of effective risk management strategies is essential to minimize occupational exposure and prevent zoonotic disease transmission [13]. Basic preventive measures include the use of gloves and protective clothing to avoid direct contact with fish mucus, frequent handwashing after handling fish or water, and avoiding eating or drinking without prior handwashing [13,98]. Regular monitoring of zoonotic agents in farmed aquatic organisms is also necessary, as prevalence varies with environmental conditions [12].

Potential occupational exposure to *Legionella* in aquaculture, fishing, and fish-processing activities may occur through the inhalation of contaminated aerosols generated during specific work operations. The likelihood of this exposure route depends on the presence of the bacterium within water systems, its persistence in environmental reservoirs such as biofilms and protozoan hosts, and the generation and dispersion of respirable aerosols. Environmental conditions that may promote the survival and proliferation of *Legionella* spp. in engineered water systems include temperatures favorable for bacterial growth, water inactivity or inadequate circulation, the accumulation of organic matter and sediments, and the development of biofilms on submerged surfaces.

Within this context, activities such as pond aeration, tank cleaning, pressure washing of facilities and equipment, fish harvesting, and the maintenance of hydraulic and water-circulation systems may constitute potential exposure scenarios when they result in the aerosolization of contaminated water. Likewise, showers, sanitation facilities, and other water distribution systems used by personnel may represent additional routes of exposure if colonized by *Legionella* and capable of generating inhalable aerosols. However, these scenarios should be regarded as hypothetical pathways of occupational exposure rather than evidence of infection or confirmed epidemiological risk within the aquaculture and fisheries sectors. The characterization of occupational risk requires specific information regarding the presence, viability, and concentration of *Legionella*, as well as the nature, duration, and frequency of worker exposure under actual operating conditions.

From a preventive perspective, comprehensive risk-management programs in aquaculture, fisheries, and seafood-processing facilities should incorporate microbiological surveillance, engineering controls, environmental monitoring, and occupational health measures. Recommended actions include periodic microbiological monitoring of water systems and environmental surfaces; routine maintenance, cleaning, and disinfection of equipment and facilities, including pumps, aerators, water-distribution systems, and storage tanks; implementation of biofilm-control strategies through physical or chemical treatments; optimization of water circulation and system design to minimize stagnation; and operational measures aimed at reducing aerosol generation during production activities [13,18,74,99,100].

Additional preventive measures should include the use of appropriate personal protective equipment (PPE), such as respiratory protection, gloves, and waterproof clothing when warranted by the risk assessment, together with worker training programs addressing hygiene practices, biological hazards, and occupational risk awareness. Particular attention should be given to personnel involved in cleaning, disinfection, and maintenance operations, who may experience greater exposure to water aerosols, especially during the use of pressure washers and high-pressure hoses. Consequently, these activities should be performed under established safety procedures and supported by appropriate training and supervision to minimize potential exposure.

Although no confirmed cases of legionellosis associated with aquaculture, fisheries, or fish-processing activities have been reported to date, the implementation of preventive and monitoring measures is consistent with a precautionary occupational health approach. Such strategies may contribute to the early identification of potential hazards, the protection of worker health, and the maintenance of microbiological safety throughout aquatic food production systems [13,74].

Finally, it is important to highlight that the development of legionellosis depends not only on the presence of bacteria but also on factors such as aerosol exposure intensity and duration, bacterial concentration, and host susceptibility [78,99]. Preventing *Legionella* colonization and proliferation in high-risk facilities is complex and requires legislative frameworks and multisectoral cooperation [101]. Although no international regulations specifically target legionellosis prevention in aquaculture or fisheries, several technical references apply to facilities with water systems capable of supporting *Legionella* growth. These include the following: 1. The World Health Organization (WHO) manual *Legionella* and the prevention of legionellosis [56]; 2. European technical guidelines for the prevention, control and investigation of *Legionella* infections [102]; 3. The World Organization for Animal Health's Aquatic Animal Health Code, which, while not specific to *Legionella*, outlines general disease prevention measures [103]; and 4. The US standard ANSI/ASHRAE 188 standard, focused on managing legionellosis risks in building water systems. In practice, risk management relies on applying and integrating these various technical references to safeguard public and occupational health [104]. Regarding the international regulatory framework for occupational health, ISO 45001 provides a global outline for implementing safety management systems, emphasizing leadership, worker participation, hazard identification, and risk assessment, among others (ISO 2018) [105].

In Latin America and particularly in Mexico, no specific Official Mexican Standard (NOM) exists for legionellosis prevention. However, several NOMs indirectly regulate microbiological monitoring and water quality control, and reduce the risk of pathogen proliferation in public, industrial, and service facilities, including NOM-127-SSA1-2021 (Water for human use and consumption; Permissible water quality limits) [106], NOM-179-SSA1-2020 (quality control of distributed water) [107], NOM-230-SSA1-2002 (sanitary requirements for public and private water supply systems) [108], NOM-245-SSA1-2010 (swimming pools water quality) [109], NOM-201-SSA1-2015 (water and ice specifications) [110], and NOM-210-SSA1-2014 (microbiological test methods for food and water for human use and consumption) [111]. In occupational safety, standards relevant to aquaculture and processing include NOM-001-STPS-2008 (buildings, premises, facilities, and areas in workplaces safety conditions) [112], NOM-017-STPS-2024 (personal protective equipment selection and use) [113], NOM-030-STPS-2009 (occupational health services) [114], NOM-015-STPS-2001 (thermal conditions) [115], NOM-033-STPS-2015 (safety conditions for performing work in confined spaces) [116], and NOM-004-STPS-1999 (safety devices on machinery and equipment in workplaces) [117]. Nevertheless, it is necessary to update the regulatory

framework to specifically address *Legionella* spp., surveillance and control in aquaculture and fisheries to strengthen occupational public health protection.

6. Conclusions

Biological hazards and diseases associated with fish and aquatic environments constitute an important public health concern due to the diversity of etiological agents and exposure pathways involved. Depending on the pathogen, transmission may occur through the consumption of contaminated aquatic products, direct contact with infected animals or tissues, or exposure to contaminated water and environments. In contrast to many fish-associated zoonotic pathogens, legionellosis is primarily acquired through the inhalation of aerosols containing *Legionella*-contaminated water, and the consumption of fish and aquatic products is not recognized as a route of transmission.

Aquatic environments and water systems capable of generating aerosols may represent potential settings for human exposure to *Legionella*. Consequently, the occurrence of *Legionella* spp. in aquaculture, fisheries, and fish-processing environments merits consideration from both occupational and environmental health perspectives, particularly when work activities involve the generation and dispersion of water aerosols. Nevertheless, in the absence of epidemiological evidence demonstrating worker exposure or establishing an association with disease occurrence, these scenarios should currently be regarded as plausible exposure pathways and potential occupational hazards rather than confirmed occupational risks.

The nature of many aquaculture and fisheries activities suggests that workers may encounter conditions conducive to exposure, especially during activities involving frequent contact with water and aerosol-generating processes. However, the identification of potential exposure pathways alone is insufficient to demonstrate the existence of occupational infection risk. A comprehensive assessment requires the characterization of environmental and microbiological conditions, including the presence, viability, and concentration of *Legionella* spp., as well as the frequency, intensity, and duration of worker exposure.

Current knowledge gaps highlight the need for further epidemiological, microbiological, and occupational health research aimed at determining the occurrence and persistence of *Legionella* spp. in aquaculture and fishery environments, identifying potential aerosol sources, characterizing exposure scenarios, and evaluating possible links between occupational exposure and infection. The generation of such evidence would facilitate the assessment of potential associations and, where appropriate, support the evaluation of causal relationships between workplace exposure and disease occurrence.

Advancing knowledge in this area is essential for improving occupational risk characterization within aquaculture, fisheries, and fish-processing sectors and for informing evidence-based strategies for environmental monitoring, epidemiological surveillance, prevention, and risk management. In parallel with ongoing research, preventive measures should be implemented in proportion to the identified hazards and the level of scientific uncertainty, thereby supporting worker health protection while avoiding unsupported assumptions regarding occupational risk.

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