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Chapter 3

Antibiotics in Chilean Aquaculture: A Review

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Abstract

Aquaculture in Chile has been practiced since the 1920s; however, it was not until the 1990s that aquaculture became an important sector here. Important species in Chilean aquaculture include salmonids, algae, mollusks, and turbot. Salmonids are the dominant species in Chilean aquaculture for both harvest volume and export value, their production reaching greater than 800-thousand tons in 2015. However, this growth has been accompanied by an increase in disease presence, requiring greater drug use to control. This increase in drug use is an environmental and public health concern for the authorities, the salmon industry itself, and the destination markets. In this chapter, we review the literature on drug use, antibiotic resistance, regulatory framework, and alternatives, with focus on Chile.

Keywords: aquaculture, Salmon, antibiotics, food safety

1. Introduction: brief history of antibiotics use in Chilean aquaculture

Antibiotics have been used to treat animals since the 1940s, which was soon followed by the appearance of resistant bacteria [1, 2]. In 1969, the House of Lords in the United Kingdom published the "Swann report," highlighting the excessive use of antibiotics in animals and its potential risks to human and animal health. The Swan report suggested that antibiotic use should be restricted and regulated. The accumulated evidence from Europe and North America supports the notion that antibiotic use should be regulated and restricted to specific clinical situations [2].

In Chile, between 1973 and 1976, the first commercial fish farming of salmonids in the Region of the Lakes was consolidated and has grown ever since [3]. In the subsequent decades, four species of salmonids of commercial importance have been cultivated in

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Chile: rainbow trout (*Oncorhynchus mykiss*), Chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), and Atlantic salmon (*Salmo salar*). In the period between 1987 and 2010, four diseases appeared in the salmon farming industry in Chile, coinciding with the beginning of antibiotic use by the industry in 1989. The diseases reported in these years were primarily due to the ectoparasite *Caligus* and bacterial kidney disease in 1987 [4–6]; the presence of *Piscirickettsia salmonis* in 1989 [4]; presenting later high mortalities due to IPN in 1997; and by the outbreaks of the infectious salmon anemia disease in the years 2002, 2007, and 2008 [5].

Sixteen antibiotics are used in animal treatments in Chile, compared to three in the United States (US) and four in Norway [7]. In the case of aquaculture in Chile, antibiotics have been mainly used in sea water Atlantic salmon farming, which accounted for 80% of the total use of antibiotics used for 2015, followed by 11% for coho salmon, 9% for rainbow trout, and 0% for Chinook salmon [8].

The consumption of antibiotics in the salmon industry in Chile has increased by 56% from 2005 to 2015, with a production increase for those years of 23.48%. The highest consumption was recorded in 2014, with a total use of 563.2 tons of antibiotics with 955,179 tons of salmonids produced. In 2016, there was a 30.66% decrease in antibiotic use compared to 2015, using a total of 382.5 tons of antibiotics to produce 727,812 tons of fish (**Table 1**) [8–11].

The most used antibiotics in the salmon farming industry in Chile are florfenicol and oxytetracycline. Florfenicol use has increased steadily since 2013 and accounted for 87% and 82.50% of the total antibiotics used in 2015 and 2016, respectively [8–11]. Florfenicol

Year	Antibiotics use annual quantity (ton)	Annual production of salmonids (ton)
2005	239.1	614.435
2006	343.8	647.302
2007	385.6	600.862
2008	325.6	630.647
2009	184.4	474.174
2010	142.2	466.857
2011	206.8	649.492
2012	337.9	836.949
2013	450.7	786.091
2014	563.2	955.179
2015	557.2	846.163
2016	382.5	727.812

Table 1. Historical consumption of antibiotics in salmon farming industry in Chile [8–11].

is mainly used in the seawater stage to control piscirickettsiosis (SRS) caused by the Gram-negative facultative intracellular pathogen *P. salmonis*. In salmonids, this epizootic disease has high mortality rates (78.9% for Atlantic salmon, 82.9% for rainbow trout, and 59.3% for coho salmon) [9].

In 2005, SRS was the most diagnosed pathology, accounting for 77.04% of the antimicrobials used in the year 2005 by the industry [9]. This trend has been maintained; 89.3% of all antibiotics used for the year 2016 have been for the control of SRS at the seawater stage followed by 6.8% for the control of renibacterosis [10].

2. Antibiotic resistance

2.1. Mechanisms of *P. salmonis* infection

In the last decade, there have been significant advances in the knowledge of *P. salmonis*, including aspects of its survival behaviors under stress conditions and genomic data. One of the first achievements has been the culture of this pathogen in the cell-free medium [12, 13]; previously, it had been necessary to develop and maintain cultures of fish cell lines. This progress has allowed the study of the physiology and behavior of this bacterium. An interesting recent finding is that *P. salmonis* can form biofilms. The development of *P. salmonis* biofilms occurs under stress conditions and salt concentrations similar to those of seawater. The biofilm matrix of *P. salmonis* is composed of exopolysaccharides and is disaggregated when treated with cellulases, which are relevant since biofilm formation might be a survival mechanism in the marine environment of this bacterium [14].

It is known that within biofilms, microorganisms are more resistant to the action of chemotherapeutics and have better survival rates under adverse conditions [15]. Recent findings have identified genes that have a role in the formation of *P. salmonis* biofilms such as the cheA gene [16]. This gene plays a key role in modulating the initiation of bacterial chemotaxis in other bacteria, such as *Pseudomonas pseudoalcaligenes* KF707 [17]. Using real-time PCR, it has been shown that cheA expression is increased during *P. salmonis* biofilm development. The results obtained in this research also suggest interaction between the formation of biofilms and the genes involved in the chemotaxis of this pathogen. Biofilm production has been reported as a potential mechanism of pathogenicity in several aquatic bacteria [18, 19]. It is very likely that the first contact with fish for the development of biofilm is produced by chemotactic responses. Chemotaxis to fish mucus has been previously reported as the first step in the development of pathogenic activity [20].

Some authors have suggested that *P. salmonis* infections begin when bacteria overgrow the skin barrier or gills [21]. In this regard, experimental infections were performed in juveniles of *Oncorhynchus mykiss* obtained from areas where the presence of SRS has never been reported, infecting them at six different entry sites. These authors found that the main entrance routes

are through skin and gills and that the oral route is not used to initiate *P. salmonis* infection of salmonids [22]. Later, this same research group performed experimental infections in coho salmon (*Oncorhynchus kisutch*). The results of cumulative mortality and survival analyses showed that the most effective entry portal was the skin, followed by intestinal intubation and finally by gill infection [23]. These findings show that *P. salmonis* can penetrate and then systemically invade the Coho salmon through the skin and mucous membranes, which appear intact at the macroscopic level, and that the skin is probably the most important site of entry of this bacterium into salmonids. These findings support the notion that biofilm formation initiates colonization of the fish, thereby activating other virulence factors such as proteases to initiate ulcerations and invasion of the organism. *P. salmonis* is not a motile bacteria and a chemotaxis process could not be activated toward the fish mucus as it happens in other fish pathogens [20]. Studies have shown that other nonmotile pathogenic bacteria can adhere to their host through their net electrostatic charges [24]. Also, nonmotile pathogens infect a host using proteases, and the genes encoding these proteases can be transmitted to nonprotease mutant strains [24].

Recent research carried out on coding and noncoding transcript during an *in vivo* infection process of Atlantic salmon with *P. salmonis* identified a common response associated with oxidation-reduction processes, endocytosis, and ion responses. In the different types of analyzed tissues, the clathrin protein, which plays a major role in the formation of coated vesicles, was significantly upregulated in infected individuals, suggesting the importance of clathrin-mediated endocytosis for the bacterial internalization. Moreover, several endocytosis receptors were repressed during the challenge [25].

2.2. Piscirickettsia salmonis resistance to antibiotics

As mentioned above, the use of oxytetracycline and florfenicol are mostly used to control *P. salmonis*. Florfenicol use almost doubled between 2013 and 2016, suggesting that the battle against this pathogen has been unsuccessful. The evolution of resistance of *P. salmonis* to antibiotics has been demonstrated. Recent isolates of *P. salmonis* (SLGO94 and SLGO95) present a higher level of resistance to antibiotics than earlier isolates (LF-89 and EM-90), suggestive of antibacterial resistance [26]. Subsequently, a large-scale study conducted to evaluate the susceptibility profiles for quinolones, florfenicol, and oxytetracycline from 292 field isolates obtained from different farm sites over a 5-year period revealed a high incidence of resistance to quinolones and early resistance to oxytetracyclines and florfenicol [27, 28].

P. salmonis genes encoding membrane-carrying proteins are upregulated in the presence of antibiotics [29]. The *P. salmonis* genome encodes efflux pumps that enable this bacterium to survive at critical concentrations of florfenicol [30]. Thus, despite the use of antibiotics, there are antibiotic-resistant (especially quinolone) bacteria in sediments near farming areas [31]. These bacteria carry plasmids that confer resistance to quinolones in marine bacteria [32]. **Figure 1** shows a proposed model for *P. salmonis* infection during the seawater stage in salmonids.

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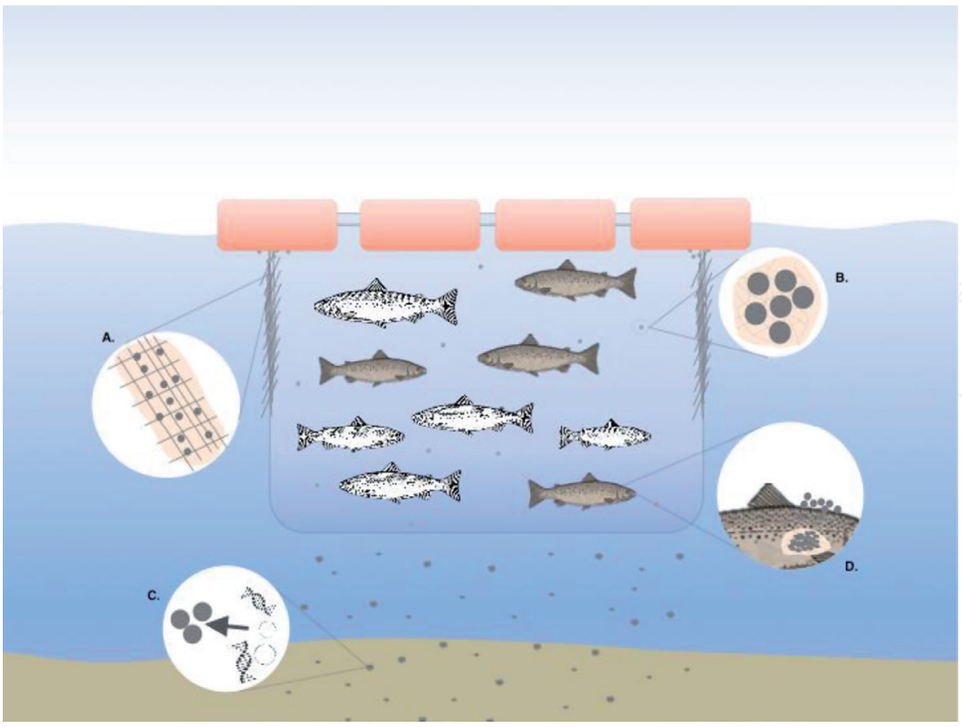


Figure 1. Model of *in situ* infection of *P. salmonis*. (A) Biofilms of *P. salmonis* in culture net; (B) biofilms in microaggregates; (C) transference of resistance genes in sediments; (D) colonization of *P. salmonis* on fish surface in captivity and onset of pathogenesis by contact with microaggregates or net. Elaborated by: Carlos Riquelme and Victor Sanchez.

3. Analysis of the regulatory framework

In Chile, in its Article 86, the Fisheries and Aquaculture law prohibits the preventive application of antimicrobials in a preventive way in aquaculture, as well as any use harmful to human health. Subsequently, there have also been the supreme decree N°319/2001, Regulation on Protection Measures for the Control and Eradication of High-Risk Diseases for Hydrobiological Species; the exempt resolution N°8228/2015 [33], the Manual of Good Practices in the Use of Antimicrobials and Antiparasitics in Chilean Salmon Farming [34]; the exempt resolution N° 5.125/ 2016, Manual on Food Safety and Certification [35]; and the Quality Assurance Program (PAC) for Fisheries and Factories Vessels [36]. This regulatory framework is jurisdiction of SENAPESCA (National Fisheries and Aquaculture Service) institution that is part of and depends on the Ministry of Economy, Development and Tourism.

According to the current legislation, only pharmaceuticals for exclusive veterinary use registered or authorized for application in hydrobiological species can be used [37]. The pharmaceutical products authorized by Servicio Agrícola Ganadero (SAG) are shown in **Table 2** [34]. The studies of effectiveness, adequate dosage, animal safety, and human food safety (toxicology) of these authorized pharmaceutical products are not available to the public or were not found.

Generic name	Trade name and registry no.	Presentation/route of administration	Registered by	Withdrawal period (degree days)	Dose mg/kg lw*/day	Tolerance level in muscle tissue (μg kg^{-1})
Oxolinic acid 80%	Reg. N°441	Powder/oral	FAV S.A.	450	20 per 10 days	100
	Litoflox Reg N°648	Powder/oral	Centrovet LTDA.	450	10–30 per 10 days	
	Bandrol Reg N°481	Powder/oral	Veterquímica S.A.	450	10 per 10 days	
Amoxicillin 50%	Amox-Feed Reg N°121	Powder/oral	Veterquímica S.A.	300	70.4 per 10 days	50
Erythromycin 50%	Vetromic Reg. N°1402-B	Powder/oral	Centrovet LTDA.	500	75–100 per 21 days	200
Erythromycin 80%	Eritofeed Reg. N°616-B	Powder/oral	Veterquímica S.A.	500	92.5 per 21 days	
	Vetromic Reg N°1803-B	Powder/oral	Centrovet LTDA	500	75–100 per 14–21 days	
Flumequine 10%	Flumepren Reg. N°79	Powder/oral	Centrovet LTDA	300	(−)	***500 ****600
Flumequine 50%	Reg. N°484	Powder/oral	FAV S.A.	300	12–25 per 10–12 days	
	Reg. N°646	Powder/oral	Centrovet LTDA	300	12–30 per 10 days	
Flumequine 80%	Reg. N°442	Powder/oral	FAV S.A.	300	20 per 10 days	
	Flox-Feed Reg. N°478	Powder/oral	Veterquímica S.A.	300	10 per 10 days	
	Flumepren Reg. N°645	Powder/oral	Centrovet LTDA.	600	12–30 per 10–15 days	
Florfenicol 50%	Florfenox Reg. N°1537	Powder/oral	Bayer S.A.	300	10 per 10 days	1000
	Veterin Reg. N°1556	Powder/oral	Centrovet LTDA.	300	10 per 10 days	
	Duflosan Reg. N°1769	Powder/oral	Veterquímica S.A.	300	10 per 10 days	
Florfenicol 50%	Duflosan L Reg. N°2264	Solution/oral	Veterquímica S.A.	100	10 per 10 days	1000
	Aquafen Reg. N°1193	Powder/oral	Intervet Chile LTDA.	200	10 per 10 days	
	Reg. N°1598	Powder/oral	FAV S.A.	300	10 per 10 days	

Generic name	Trade name and registry no.	Presentation/route of administration	Registered by	Withdrawal period (degree days)	Dose mg/kg lw*/day	Tolerance level in muscle tissue (μg kg^{-1})
Oxytetracycline hydrochloride 20%	Terrivet F200 Reg. N°2252	Suspension for injection	Veterquímica S.A.	1060	**20	200 (−) Tetracyclines
Oxytetracycline 50%	Terrivet Reg. N°149	Powder/oral	Veterquímica S.A.	600	75 per 15 days	
Oxytetracycline 80%	Terrivet Reg. N°485	Powder/oral	Veterquímica S.A.	600	75 per 15 days	
	Reg. N°1595	Powder/oral	FAV S.A.	600	55–82 per 10 days	
	Zanil Reg. N°1380	Powder/oral	Centrovet LTDA.	600	75 per 10 days	
Oxytetracycline 40%	Reg. N°309	Powder/oral	Laboratorio Veterinario Quimagro S.A.	600	13.57–20.75 per 10 days	

*lw = live weight.

**mg/kg/lw.

***Trout.

****Other salmonids.

Table 2. Antimicrobials for salmonids authorized by the Veterinary Medicines Registry (SAG) [34].

The Chilean authority supervises the use of pharmaceutical products in hydrobiological species, and in accordance with the provisions of the general and specific health programs, therapeutic treatments applied to populations of hydrobiological species should be prescribed by a veterinarian and the application of antimicrobials for prophylactic purposes is prohibited. Before the application of the antimicrobials, fish samples should be obtained for subsequent confirmation of the diagnosis by laboratory analysis [37]. Farming facilities should keep records of antimicrobial treatments performed and antimicrobial treatments should be reported monthly through the Aquaculture Inspection System (SIFA) [34].

Extralabel antimicrobials can be prescribed by a veterinarian when the health of an animal is at risk, there is danger of death, or there is suffering of the animal; or when one of the following is fulfilled: dosage, timing, duration of treatment or route of administration for a registered product does not obtain the expected response; the product is temporarily unavailable on the market; or there is no registered product to treat a diagnosed condition [34].

The Manual on Food Safety and Certification (resolution N° 5.125/ 2016) describes the norms and procedures that allow to guarantee the sanitary quality of the fishery and aquaculture products destined for international markets along the whole productive chain. Regarding

the procedures for the control of residues of pharmaceutical products, each farm facility must demonstrate (and issue a declaration of guarantee) that the concentrations of residues of pharmaceutical products in fish do not exceed the limits established by the Chilean authority [35].

The analysis must be carried out in authorized laboratories, according to the Methods of Analysis of Residues of Pharmaceutical Products and Contaminants for Export Fishery Products. If the maximum allowable limits are not met, the withdrawal period should be extended and a new sampling should be carried out [35].

The government of Chile also maintains a Program of Surveillance and Control of Piscirickettsiosis, in which monitoring system and the application of control measures are established for this disease [38]. Upon request, the Chilean authority issues fish farming centers a certificate stating that the fish are free of antimicrobial and/or antiparasitic treatments [39]. The Quality Assurance Program (PAC) is a voluntary certification program, based on the concept of hazard analysis and critical control point (HACCP), which applies only to fishing plants and factory vessels. This program, however, is mandatory for all companies that are authorized to export to the European Union and the United States. The Chilean authority must approve the quality assurance plan for the industry and supervise its subsequent operation [36]. The administrative procedures, work guides, and specific requirements of this program (PAC) are not publicly available.

The main Chilean salmon markets are the US and Japan. In the case of the US, the Food and Drug Administration (FDA) is in charge of regulating the use of antibiotics in fish, primarily through its regulation 21 Code of Federal Regulations (CFR) 123 "Procedures for the Safe and Sanitary Processing and Importing of Fish," which aims to ensure the safe and sanitary processing of fish and fishery products (seafood), including imported seafood [40]. The regulation mandates the application of HACCP principles to the processing of seafood as a preventive system of hazard control that can be used by processors to ensure the safety of their products to consumers. For the control of drugs for use in food of animal origin, direct medication or for addition to feed must be approved, conditionally approved, or index listed by the FDA (Federal Food, Drug, and Cosmetic Act Section 512) [41].

Under certain conditions authorized by FDA, unapproved new animal drugs may be used in conformance with the terms of an Investigational New Animal Drug (INAD) application (21 CFR 511) [42] and FDA's Center of Veterinary Medicine (CVM) guide 1240.3025. When a drug is approved by CVM, the condition of the approval is listed on its label or in the labeling (21 CFR 514.1) [43]; this condition specifies the species for which the drug is approved for use, indications for use, dosage regimen, and other limitations such as route of administration and withdrawal time. Labeled withdrawal times must be followed to ensure that no harmful drug residues are present in the edible tissue of the animal when harvested for human consumption; tolerances for some drug residues in the edible tissue have been established [44].

Relatively few drugs have been approved for aquaculture in the US (**Table 3**). This has led to the inappropriate use of unapproved drugs, general-purpose chemicals, or approved

Antibiotic	Dose	Approved for:	Route of administration	Tolerance level in muscle tissue	Withdrawal period
Terramycin 200 (oxytetracycline dehydrate)	2.5–3.75 g/100 lb. of fish/day Maximum dose = 8.33 mg/kg/day/10 days	Salmonids: For control of ulcer disease caused by *Haemophilus piscium*, furunculosis caused by *Aeromonas salmonicida*, bacterial hemorrhagic septicemia caused by *A. liquefaciens*, and pseudomonas disease, for control of mortality due to cold-water disease associated with *Flavobacterium psychrophilum*. Freshwater-reared *Oncorhynchus mykiss*: For control of mortality due to columnaris disease associated with *Flavobacterium columnare*	Administer in mixed ration for 10 days; do not liberate fish or slaughter fish for food for 21 days following the last administration of medicated feed.	2 ppm (As the sum of tetracycline residues)	21 days for disease control in salmonids 7 days for marking skeletal tissue in Pacific salmon
OxyMarine Oxytetracycline HCL soluble powder-343, TETROXY Aquatic	200–700 mg oxytetracycline/L of water for 2–6 h	For marking of skeletal tissues in finfish fry and fingerlings as an aid identification	Immersion	2 ppm (as the sum of tetracycline residues)	21CFR 556.500
Sulfadimethoxine/ormetoprim combination Romet-30		For control of furunculosis in salmonids (trout and salmon) caused by *Aeromonas salmonicida*	Medicated feed	0.1 ppm for each drug (21 CFR 556.490)	42 days (21 CFR 558.575)
Florfenicol Aquaflor	10 mg/kg fish per day for 10 consecutive days	For the control of mortality in freshwater-reared salmonids due to cold-water disease associated with *Flavobacterium psychrophilum*	Medicated feed	2 ppm in salmon muscle/skin	15 days

Table 3. FDA aquaculture approved drugs, route of administration, and tolerance levels [40, 44].

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drugs in a manner that deviates from the labeled instructions [44]. Studies establishing the effectiveness, adequate dosage, animal safety, and human food safety (toxicology) of these approved drugs are available to the public [45].

In the case of Japan, fishery products are regulated by the Food Sanitation Act and the Food Safety Basic Act. The authorities involved with in the Food Sanitation Act are as follows: the Office of Import Food Safety; Inspection and Safety Division; Pharmaceutical and Food Safety Bureau; Ministry of Health, Welfare and Labor; and the Department of Food Safety. The purpose of the Food Sanitation Act is to prevent the occurrence of health hazard arising from human food so as to contribute to the protection of people health by conducting regulations and measures deemed necessary, from the view point of public health and for securing food safety [46].

The purpose of the Food Safety Basic Act is to promote comprehensive measures to secure food safety by laying down the basic principles of safety for food, defining the responsibility of the government, local authorities, and food-related businesses, clarifying the role of consumers, and establishing basic policies for developing measures. The authority concerned is the Consumer Affair Agency [46].

Antibiotic residue concentrations for edible products from food-producing animals are determined based on jurisdictional-specific regulations that result in the determination of a tolerance or maximum residue level (MRL) for specific drugs in a specific tissue for specific animal species and based on toxicological assessments. This index estimates the amount of substance in food that can be ingested over a lifetime by humans without significant risk to health [47]. There are notable differences among MRLs or tolerances set by the different agencies regarding the two antibiotics most used in Chilean salmon farming (**Table 4**).

Many methods have been developed for analysis of antibiotics in fish. HPLC and mass spectrometry (HPLC-MS/MS) is the most sensitive method for the detection of these antibiotics and is currently regarded as the tool of choice for analysis of antibiotic residues in

Antibiotic	Europe (μg kg^{-1}) MRL	[e]Chile (μg kg^{-1})	[f]USA tolerance (ppm)	[g]Japan (ppm)
Oxytetracycline	[a]100	200	[c]2	[d]0.2
Florfenicol	[b]1000	1000	1	0.2

[a]508/1999/EC.

[b]1322/2001/EC.

[c]As a sum of tetracycline residues [44].

[d]Calculated as oxytetracycline.

[e][34].

[f][44].

[g][48].

Table 4. Antibiotics used in Chilean salmon farming and their maximum residue limits in salmonids (MRLs).

animal-derived food [47, 49], having a limit of detection (LOD) in fish of 10.3 ng/l [50] and a limit of quantification of 20 ng/l for tetracyclines [51].

4. Alternative antibiotic treatment in salmon farming

Fish are considered as the earliest class of vertebrates to have both innate and adaptive immunity, though the latter defense mechanism is not as elaborate as in higher vertebrates. Unlike in mammals, the alternative complement pathway in teleosts is relatively high and can mediate the lysis of target erythrocytes from several species. These features, along with their potential to function at varying temperatures, suggest that the complement system is a powerful defense mechanism in fish [52–54], and they are in constant interaction with their surroundings and therefore could easily encounter potential pathogens. In the wild, fish can protect themselves using innate defense mechanisms (either constitutive or responsive) [52].

The various alternatives to the use of antibiotics can be classified according to the action toward the pathogen or host. Pathogen-directed strategies include inhibitors of growth and virulence genes, antibacterial compounds, and the phage therapy. Host-directed strategies include the improvement of health, stress prevention, stimulation of the defense system, and selective breeding for disease resistance [55].

One of the first lines of defense against bacterial infection is the withholding of nutrients, termed nutritional immunity. The most significant form of nutritional immunity is the sequestration of iron [56]. Recent studies have detected a relationship between iron transporter glycoproteins and *Salmo salar* susceptibility to pathogens [57, 58]. In salmonids, an iron transporter glycoprotein has been identified as a vaccine enhancer [59]. In vertebrates, it has been shown that iron transporter glycoproteins exert antibiofilm therapeutic [60, 61] and antimicrobial activity by binding to iron, thereby preventing its use by bacteria [61–64] and thus causing alterations in the bacterial wall and, ultimately, death. Because of its cationic nature, this glycoprotein binds to the lipopolysaccharides of Gram-negative bacteria, thereby attenuating those proinflammatory processes induced by bacterial lipopolysaccharides [63]. Among alternative sources of bioactive compounds, ingredients or products derived from marine algae show great potential for use in aquaculture [65]. Rainbow trout–supplemented diets with phytopharmaceutical of herbal and macroalgal origin have improved resistance against *P. salmonis* [66].

Several bacteriophages have been isolated against the following pathogenic bacteria, *Edwardsiella tarda, Edwardsiella ictaluri, Lactococcus garvieae, Pseudomonas plecoglossicida, Streptococcus iniae, Flavobacterium columnare, Flavobacterium psychrophilum, Aeromonas salmonicida, Aeromonas hydrophila, Vibrio anguillarum, Vibrio harveyi, and Vibrio parahaemolyticus*, and their potential to be used as a therapeutic agent has been studied by several researchers [67]. In salmonids, *Flavobacterium psychrophilum* phages have shown protection

against bacterial cold water disease *in in vitro* conditions. Each phage isolate rarely infected *F. psychrophilum* strains other than the strain used for its enrichment and isolation. Some bacteriophages decrease mortality from intraperitoneal injection of their host strain when added together with the bacteria at a ratio of 10 plaque-forming units per colony-forming unit [68].

Promising results have been obtained in laboratory studies. However, high concentrations of bacteriophages in seawater can induce bacterial genetic variation. This occurs through mutation and bacteriophage-mediated horizontal transmission of genetic material between different bacteria mediated by bacteriophages [5, 69]. The use of phages can also influence bacterial community dynamics and ecosystem biogeochemistry. These influences differ depending on whether phages establish lytic, chronic, or lysogenic infections. The impacts of lysogeny are well studied at the cellular level, but ecosystem-level consequences remain underexplored [70].

Probiotics have been credited for producing improved nutrition, health benefits, reduced disease incidence, improving growth, health status, immunity, feed conversion, microbial balance, and water quality, as well as food production in an environmental-friendly way [71–73]. Probiotics in aquaculture can be live or dead preparations, including cellular/extracellular components of the microorganism(s), administered either as a feed supplement or to the rearing water. Probiotics can be used to control a range of bacterial pathogens in various fish species [69]. For example, rainbow trout (*Oncorhynchus mykiss*) were protected against *Aeromonas salmonicida* and *Yersinia ruckeri* when administered with dietary *Carnobacterium maltaromaticum* and *C. divergens* [72]. The efficacy of *Carnobacterium* sp. at reducing diseases caused by *A. salmonicida*, *V. ordalii*, and *Y. ruckeri* in salmonids has also been demonstrated [72]. However, there is no solid knowledge regarding the potential of probiotic against *P. salmonis*.

The Food and Agriculture Organization of the United Nations (FAO) has now highlighted the use of probiotics in aquaculture as a means of improving the quality of the aquatic environment [72]. However, concerns have been voiced about the possible acquisition of antibiotic resistance and virulence genes via horizontal gene transfer, which might lead to safety problems if using live probiotics in an open aquatic environment. Probiotics can also affect host tissue and result in severe cell damage. To avoid this, probiotic strains must be recognized as safe for the cellular integrity of the host [72].

In the aquaculture industry, vaccination strategies include traditional inactivated and attenuated vaccines, as well as next-generation vaccines comprising recombinant, subunit, vectored, genetically engineered, DNA and peptide vaccines, reverse vaccinology, plant-based edible vaccines, and nanovaccines [74]. Current vaccination protocols for *P. salmonis* include whole cell, inactivated and adjuvant vaccines for injection (primary immunization), followed by oral boost (where the timing of boost delivery is determined by measuring circulating antibody levels against the pathogen). Live vaccines and DNA vaccine studies have been unsuccessful under laboratory conditions. There are more than 25 different vaccines against SRS that are available in the Chilean market. These vaccines confer good short-term protection against disease and mortality but are inefficient at conferring long-term protection, or the duration of protection is insufficient to protect the fish throughout their economic life [75–77].

5. Conclusions

Veterinarians in charge of the salmon industry in Chile have used large quantities of antibiotics relative to its production volumes. In the years of highest production, an average of 600 g ton^{-1} produced was used. The antibiotics used by this industry are florfenicol and oxytetracycline for the control of *P. salmonis* at the seawater stage; studies have demonstrated the resistance of this pathogen to quinolones, oxytetracycline, and florfenicol, as well as their mechanisms of resistance.

There are 12 different types of generic and 25 branded antimicrobials authorized for use in salmonids in Chile, with no specifications related to pathogens or diseases. This is in contrast to the US situation, where the FDA has approved just four antibiotics for specific uses and against certain pathogens.

The studies of the effectiveness, adequate dosage, animal safety, and human food safety (toxicology) of the authorized pharmaceutical products, as established by the Chilean authority, are not available to the public or were not found. This was also the case for the administrative procedures, work guides, and specific requirements of the Quality Assurance Program (PAC), whereas the effectiveness and toxicology studies of the FDA-approved antibiotics are freely available online. This absence of Chilean regulation and antibiotic data is concerning. To avoid chemical hazards and ensure food safety, we propose that a mandatory legal framework based on international regulations is needed in Chilean aquaculture. Antimicrobial treatment is required for an efficient production of animal products; however, antibiotics should never be used as a substitute for proper nutrition and hygiene management.

The alternatives to the use of antibiotics in Chilean salmon farming, such as the use of nutritional immunity, phytopharmaceuticals, probiotics, antimicrobial peptides, and selective breeding for disease resistance, require advanced research with *in vivo* studies. Although several vaccines have been authorized, this remains an inefficient strategy for the control of pathogens in aquaculture.

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