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Streptococcus iniae in aquaculture: a review of pathogenesis, virulence, and antibiotic resistance

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ABSTRACT

One of the main challenges in aquaculture is pathogenic bacterial control. *Streptococcus iniae* stands out for its ability to cause high mortality rates in populations of commercially important fish populations and its recent recognition as an emerging zoonotic pathogen. The rise in identifying over 80 strains some displaying antibiotic resistance coupled with the emerging occurrence of infections in marine mammal species and wild fish underscores the urgent need of understanding pathogenesis, virulence and drug resistance mechanisms of this bacterium. This understanding is crucial to ensure effective control strategies. In this context, the present review conducts a bibliometric analysis to examine research trends related to *S. iniae*, extending into the mechanisms of infection, virulence, drug resistance and control strategies, whose relevance is highlighted on vaccines and probiotics to strengthen the host immune system. Despite the advances in this field, the need for developing more efficient identification methods is evident, since they constitute the basis for accurate diagnosis and treatment.

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1. Introduction

Aquaculture has grown significantly, becoming a crucial component to the objectives outlined in the 2030 Agenda for Sustainable Development [1]. For this industry, one of the main goals is optimizing of aquaculture operations to minimize environmental impacts, aided by monitoring and regulating report on ecological, social, and economic aspects of aquaculture development [2].

Within this context, one of the main challenges of aquaculture production is diseases prevalence [3]. The streptococcal disease caused by the bacterium *Streptococcus iniae* is regarded as having a major impact on the aquaculture sector because it kills economically valuable fish species at higher rates [4,5], causing considerable worldwide economic losses [4–6]. Furthermore, this pathogen can affect terrestrial organisms and has been classified as an emerging zoonotic pathogen [6–8].

Streptococcus iniae (ATCC® 29178™) was initially isolated from subcutaneous lesions found in a freshwater dolphin species from the Amazon River (*Inia geoffrensis*) under captive conditions [9,10]. This is a Gram-positive pathogenic bacterium of spherical shape [11] exhibits a beta-haemolytic nature and demonstrates adaptability to anaerobic conditions [12]. This pathogen finds favourable conditions for its proliferation in marine and freshwater environments, especially in tropical and subtropical regions [4,13–19]. To date, more than 80 strains have been identified and isolated from different species of cultured fish in various geographic areas [13,20–22].

In 2020, the Ministry of Agriculture and Rural Affairs of China classified streptococcosis as a contagious invasive disease (Class II) due to its severity [23]. Recent outbreaks of this infection were documented in aquaculture farms in Brazil and Mexico [24,25]. Although it is a significant pathogen in aquaculture, recent research has reported streptococcosis presence caused by this bacterium in marine mammals and fish, indicating that it could pose a risk to wild populations [26–29]. Moreover, genetic factors have been recently facilitating the emergence of variations that allow colonizing of new host tissues or even taxa, evasion strategies from the immune system in vaccinated hosts [21] besides antibiotic-resistant strain emergence [30–32].

The negative impact of *S. iniae* on the aquaculture industry is a potential harm to human health and wild aquatic organisms, coupled with the diversity and adaptability of the strains, posing a significant

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challenge for its control. Therefore, the present study aims to provide a more comprehensive understanding of the factors influencing the bacterium prevalence and behaviour through bibliometric analysis and a review of existing scientific literature.

2. Trends in research associated with *Streptococcus iniae*

The bibliometric analysis makes it possible to understand patterns, diversity, productivity, and the effects of a specific issue and with this knowledge facilitate the development of effective approaches [33]. For this reason, a search was conducted on the ScienceDirect platform for research articles using theVOSviewer platform (https://www.vosviewer.com/) [34], specifically with the name of the microorganism (*Streptococcus iniae*) as the keyword within the timeframe spanning from 1999–2023. A total of 1,737 results were obtained, encompassing both original articles and reviews. From this dataset, a bibliometric analysis was performed [34].

Figure 1 displays the cluster map – a product of the co-occurrence analysis of keywords within the 1,737 articles. This analysis identified a set of 3,724 keywords, among which 211 showed co-occurrences more than five times across the various scrutinized documents. Larger nodes mean higher keyword frequencies in the analysed documents, while smaller nodes indicate fewer occurrences. Furthermore, the interconnecting lines and their thickness represent the relationships between keywords with thicker lines indicating a stronger association [33].

The cluster map highlights a predominant trend in research, which focuses on understanding the innate immune response of fish to bacterial exposure. The comprehension of the immune system reaction to the presence of pathogens is essential for designing effective strategies, such as vaccination, to provide specific immunity [35,36].

Concomitantly, research on gene expressions related to inflammatory processes, cytokine formation activation, and genes associated with bacterial infections also stand out. These studies are associated with both the immune response and vaccination. As observed some formalin-killed cells based vaccines induce the activation of proinflammatory factors that regulate the expression of other cytokines. These cells are notably fundamental in regulating a positive inflammatory response [37–39], making the identification of the activation of these genes a valuable indicator.

Additionally, the cluster map shows that research focused on the development of control strategies, such as antimicrobial peptides, is less common compared to studies related to the organism immune response. However, studies have highlighted their advantages and applications [40–42], whose low likelihood of inducing bacterial resistance stands out. The reason is that by interacting with the bacterial cell membrane, its charge is neutralized and subsequently induces cell death by penetrating the membrane [43]. Notably,

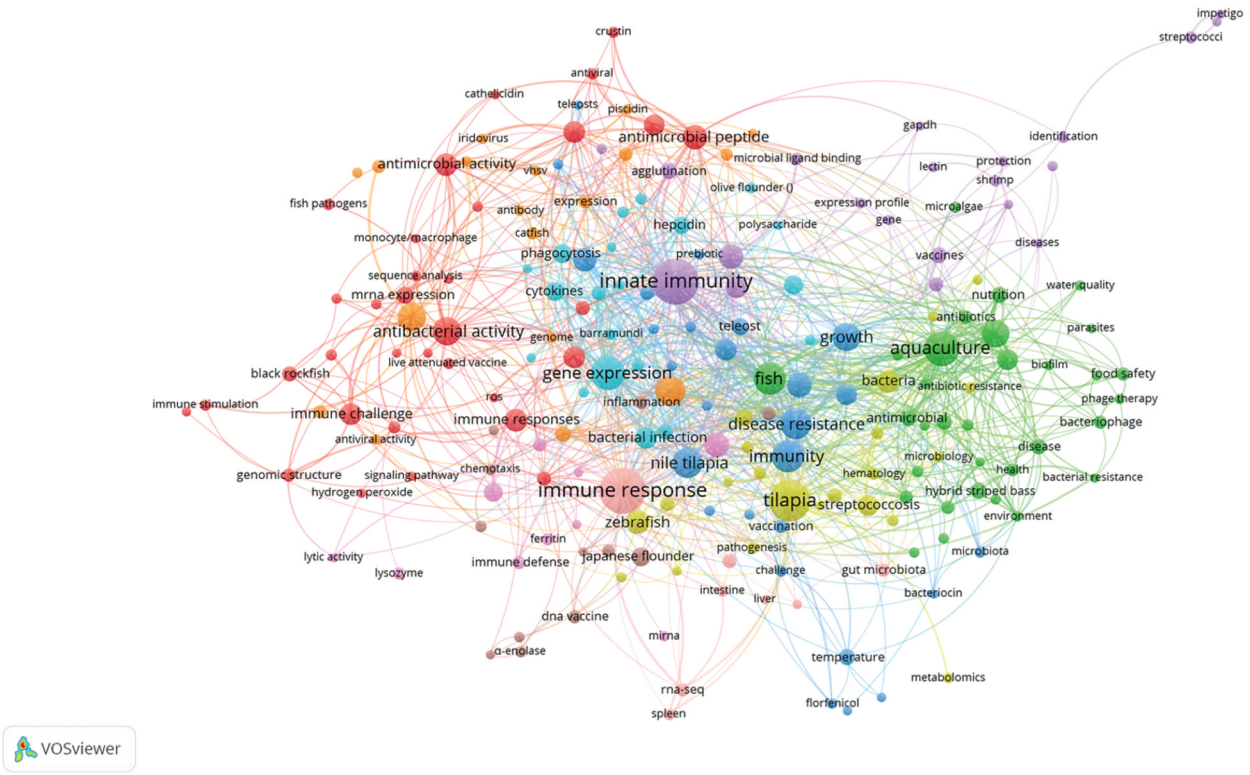

Figure 1. Bibliometric co-occurrence analysis of research trends related to *Streptococcus iniae* cluster map.

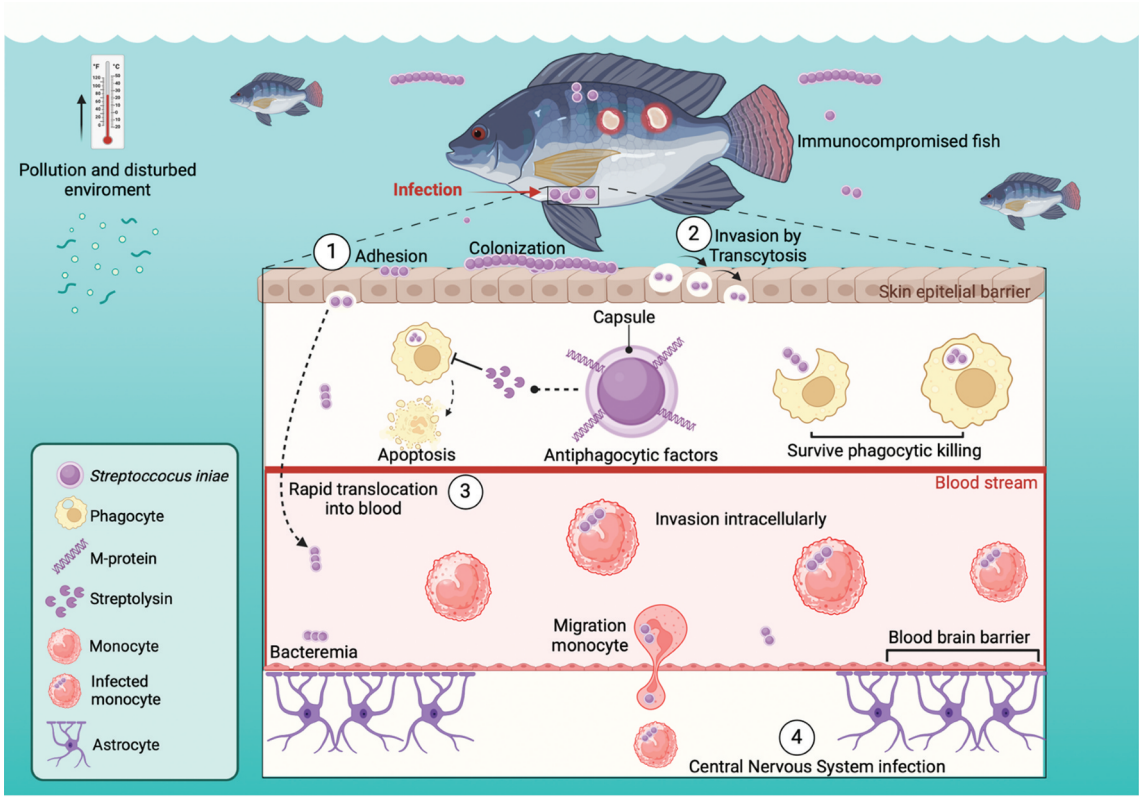

Figure 2. Graphic representation of *Streptococcus iniae*, pathogenesis process in fish. When a pathogenic microorganism gets into a susceptible host, stress factors interact and infection occurs: (1) recognition and adhesion; (2) invasion through transcytosis; (3) evasion and translocation; (4) the pathogen has successfully invaded the host at this stage and proliferates (adapted from Gnanagobal & Santander, 2022). Image created using Biorender.

many of these peptides exhibit low toxicity to organisms [44,45], posing a stable and soluble molecular structure [46].

3. Cursive typography pathogenesis in fish

The pathogenicity of a microorganism refers to its ability to cause harm to the host [47,48]. Currently, studies on a microorganism pathogenicity emphasize the pathogen recognition, invasion, proliferation and survival within the host, as well as the host's response to infection [29]. In this context, the following description outlines the streptococcal process infection in fish, known as streptococcosis, from the perspective of the pathogen *Streptococcus iniae,* illustrate in Figure 2.

(1) *Host Recognition and Adhesion*: The first step in the interaction between pathogen and host is adhesion to the host surface [45]. Key molecules in this process are bacterial adhesins, present as proteins or polysaccharides with some secreted in the form of polysaccharide capsules; adhesins are located on the bacterial cell wall and facilitate the attachment of the microorganism to host cells [46,49,50]. This binding triggers complex signalling cascades in both the pathogen and host, which can lead to extracellular colonization and invasion of underlying cells [51]. The adherence capacity of *S. iniae* to fish epithelium is attributed to the production of M-family adhesins, encoded by the *simA* gene, which are responsible for adhesion, invasion, and resistance to phagocytic destruction [46]. However, this adhesion capacity is enhanced by the mutant capsule Δ*cpsD*, where the capsule operon contains the *cpsD* gene, necessary for capsule polymerization [52]. Additionally, *cpsD* facilitates the export of repeated sugar units to the cell surface in a later stage of capsule formation, reducing the host phagocytic capacity, since macrophages cannot recognize surface molecules [52,53].

(2) *Invasion*. The pathogen spreads and colonizes various organs or tissues, sustaining the infection cycle. *Streptococcus iniae* possesses the capability to invade intracellular epithelial tissue and translocate into internal ones through the process of transcytosis intrinsic to host cells. This process occurs without being immediately recognized as cellular damage and takes approximately 30 min. After the penetration of the pathogen in epithelial cells, its dissemination via internalization within fish

macrophages may transpire [46] and facilitate the potential invasion of other tissues or cells, while the nutrients to sustain its metabolism are simultaneously acquired [47]. Thus, as documented *S. iniae* strains form biofilms under both *in vitro* and *in vivo* conditions, and this may play a crucial role in colonization, invasion, and persistence process of the bacteria in the host [17,54–56]. However, the understanding of these processes is still in its early stages, since studies on this matter are limited.

(3) *Evasion and Translocation*. During this stage, the pathogen resides in the host intracellular environment, avoiding or deactivating the immune response by inducing apoptosis in the host's macrophages. This process was reported in a study as the "Trojan Horse Effect", because *S. iniae* cells can enter and multiply within macrophages, causing their death through apoptotic processes. In this process, the hydrophobic cell walls present in the bacterial capsule allow *S. iniae* to resist phagocytosis by macrophages, facilitating its rapid dissemination to other tissues through the bloodstream, where it can also hijack monocytes to evade other host defence mechanisms. As a result, it gains access to the central nervous system [57].

(4) *Proliferation and Host Survival*. Ultimately, once the pathogen has successfully invaded the host, it utilizes the host cells for replication and survival, and proliferate within the host [58]. During this phase, the bacteria can establish themselves in various organs, such as the fish brain, where antigens encounter difficulties in crossing the blood-brain barrier, as well as in the intestines or bones [59–61].

Indicative symptoms of *S. iniae* infection in fish include exophthalmia, erratic swimming, lethargy, dorsal rigidity, meningoencephalitis, ulcers, septicaemia, ascites, haematomas, and consequently, high mortality [61–63]. Mortality in fish is often attributed to meningoencephalitis, triggered by the systemic bacterial dissemination through the bloodstream and vital organs, such as liver and kidneys [10].

4. Virulence factors

The pathogenesis of a bacterium is determined by the degree of virulence [44]. Virulence, in turn, is determined by the production of extracellular molecules called virulence factors [17]. In the case of *S. iniae*, virulence is governed by various mechanisms, including resistance to phagocytic action, damage inflicted on host cells, and its adhesion and invasion strategies [46]. These factors trigger responses from both the innate and adaptive immune systems, resulting in an increase in the production of specific serum antibodies [64]. Additionally, the host experiences positive regulation of chemokines, defensins, and cathelicidins [65]. Furthermore, the expression of genes is activated when involved in processes, such as proteolysis, phagocytosis, apoptosis, and activation of the NF-kB (Nuclear Factor-kappa B) protein complex. The latter is implicated in the cellular response to stress factors. Additionally, a rapid adaptive immune response has been observed mediated by T cells [66].

Nevertheless, various *S. iniae* serotypes have been observed showing differences in their virulence factors. For instance, some strains isolated from human samples exhibited dissimilarities compared to strains isolated from marine hosts [67]. Similarly, variations in virulence were noted among strains involved in infectious events within the same locality at different times [68]. Such variations pose challenges in identifying and standardizing methods to combat it [69].

Currently, virulence factors are understood to encompass surface proteins, capsular polysaccharides, certain extracellular products secreted by the microorganism, and extracellular nucleases [70]. Virulence factors become active once the microorganism has disseminated within the host organism, triggering the production of various enzymes. Figure 3 illustrates the presently acknowledged virulence factors.

Protein M (SiM) plays a pivotal role in virulence; these spiral-shaped proteins have a molecular mass ranging from 53 to 59 kDa and are responsible for adhering to epithelial cells, facilitated by the LPXTG anchoring motif (leucine-proline-X-threonine-glycine; X represents any amino acid). Additionally, they contribute to macrophage resistance – the simA gene is responsible for producing these proteins – and their expression is controlled by the regulatory protein mgx [71].

The C5a peptidase is a 123 kDa protein that hydrolyzes the complement chemotactic factor of neutrophils, thereby impairing the infected host ability to combat the infection. This action essentially hides the microorganism from the host-produced neutrophils [46].

The IL-8 protease production of *S. iniae* is anchored to the cell envelope through the LPTXG motif. This 1,631-amino acid protein can degrade Interleukin-8 (IL-8) produced by the host as a defence mechanism, providing the bacterium with resistance against neutrophils and facilitating disease dissemination [72].

The production of cytolysins, specifically streptolysin S, enables *S. iniae* to affect erythrocytes, neutrophils, lymphocytes, and certain types of host tissues. The *sagA* gene encodes these proteins, and its regulation is governed by a two-component signal transduction system known as sivS/R [73,74]. Moreover, *Streptococcus iniae* can cause synergistic lysis of erythrocytes through a 27

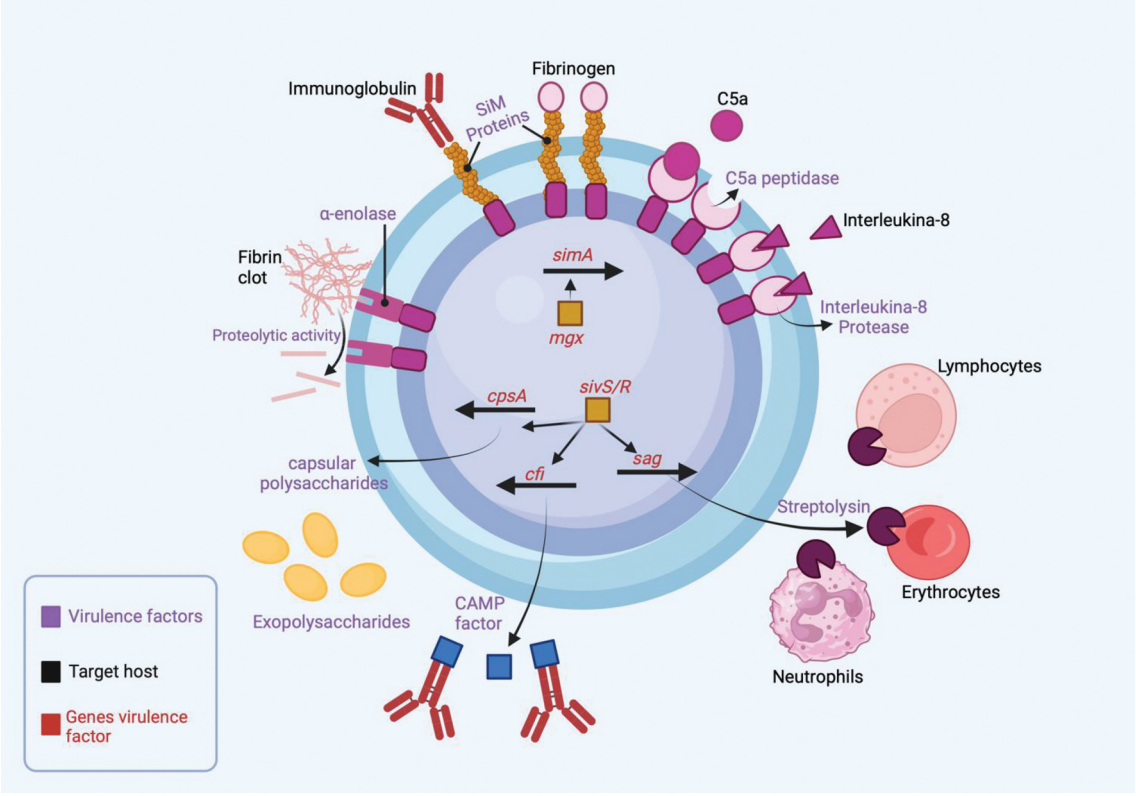

Figure 3. Schematic representation of the bacterium *Streptococcus iniae* and the presence of its virulence factors. The image depicts regulatory genes involved in the expression of virulence factors within the cell, as well as the extracellular virulence factors. At the centre, the expression **mgx** regulates the production of the **SiM** protein (*simA)* which binds to immunoglobulin and fibrinogen. Peptidase C5a and interleukin-8 protease alter phagocyte signalling by degrading chemokines. The sivS/R system regulates the production of streptolysin S cytotoxin production. Streptolysin S lyses lymphocytes, erythrocytes, and neutrophils; sivS/R also regulates the CAMP factor gene, **cfi**, which binds to immunoglobulin via the **Fc** region. Capsular polysaccharide synthesis (*cpsA*; ***CPS***) is controlled by sivS/R. The excess production of exopolysaccharide contributes to highly viscous growth. α-enolase degrades fibrin clots and promotes their dissemination (adapted from Baiano & Barnes, 2019). Image created using Biorender.

kDa peptide encoded by the cfi gene and regulated by the sivS/R system. This interaction occurs with an extracellular protein (CAMP factor) like the CAMP factor of *Staphylococcus aureus*, resulting in erythrocyte lysis. Furthermore, this factor has shown to be capable of binding to immunoglobulins, which could potentially serve as a target for detecting the microorganism within the host [53].

Lastly, one of the most successful virulence factors is the formation of capsular polysaccharides by the microorganism, since they aid in evading phagocytosis. The *sivS/R* system regulates the production of streptomycin S, the CAMP factor and capsule polysaccharide production, which are mediated by the *cpsA* operon [75]. Another virulence factor on the cell surface is the polysaccharide deacetylase encoded by the *Pdi* gene, this polysaccharide gives the bacterium resistance to lysozyme destruction and provides it with the ability to adhere to and invade epithelial cells [76]. Furthermore, the excessive production of exopolysaccharides leads to increased viscosity and the ability of *S. iniae* to traverse tissues by activating plasminogen through the action of α-enolase and also contributes to the microorganism's virulence. These factors enable subsequent migration into the host organism [8].

5. Contact based infection in human

The first reported cases of *S. iniae* infections in humans occurred in 1996 [77] and it was classified as a zoonotic pathogen at the International Conference on Emerging Infectious Diseases in 2000 [78]. Until 2021, 25 cases of infection were reported in humans. However, the actual number is estimated to have been higher due to misidentifications [59], and traditional methods do not allow differentiation between groups of bacteria with phenotypic similarities [79]. Human infections have mainly occurred in susceptible older individuals with multiple conditions such as diabetes mellitus, chronic rheumatic heart disease, cirrhosis, and immunocompromised patients [80]. Cases have been primarily reported in North America, the Middle East, the Asian-Pacific region, and Asia [7,77].

The infection is attributed to percutaneous exposure to carrier fish during the selection process for commercialization [66,80]. Manipulation of these organisms can cause soft tissue injuries through

which the bacteria enter, leading to bacteraemic cellulitis in the hand [81] and other more serious conditions, such as endocarditis, meningitis, arthritis, sepsis, pneumonia, osteomyelitis, lymphangitis, bloodstream infection, and toxic shock [81–83]]. Although the bacterial infection was once considered rare, predictions considered a possible increase in cases due to improvements in detection and diagnostic methods, as well as aquaculture global expansion [7]. Until the 1990s, detection methods relied on serological tests [84]. Subsequently, Polymerase Chain Reaction (PCR) began to be used with the advancement of DNA analysis [85,86], along with other methods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In addition, qPCR assays have been recently developed as another identification alternative, showing 100% specificity for DNA extractions from pure *S. iniae* cultures and from the tissue of infected fish [87].

A recent study demonstrated that *S. iniae* laboratory identification poses a significant challenge, since traditional biochemical tests may yield inaccurate results. In addition, resistant strains to antibiotics exist, leading to consider this pathogen becoming an emerging challenge [3].

In the meantime, some precautionary measures can be taken when handling or fileting farmed fish to prevent *S. iniae* infections in older adults and immunocompromised individuals [77].

6. Risks of antibiotic use

Poor management of sanitary conditions in aquaculture leads to pathogenic bacterial proliferation [88,89]. Thus, the need to use various antibiotics for their control arises [90]. The use of antibiotics in aquaculture is highly influenced by the legislation implemented in each country. While certain medications are prohibited in the European Union, permissible maximum concentrations vary in other countries, resulting in a lack of uniformity in treatments. This variation is based on the organism and the conditions under which they are applied [91]. Chemicals, including fluoroquinolones, furazolidone, nitrofurazone, dimetridazole, and chloramphenicol are frequently permitted during infectious outbreaks [92].

As observed in white shrimp *(Penaeus vannamei)* intensive production in Vietnamese farms, the continuous use of antibiotics have led to the establishment of resistant strains [93]. These researchers examined *Vibrio* species' resistance to various antibiotic concentrations, including streptomycin, gentamicin, and ciprofloxacin. After three months of continued usage, the quantities of harmful bacteria in certain cases reached 104–106.6 colony forming units (CFU)/mL.

Similar findings were reported in another study conducted on *P. vannamei* farms. These farms obtained bacterial isolates including, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio fluvialis*. As well as algae, such as *Shewanella*, *Aeromonas hydrophila*, and *Aeromonas salmonicida*. These strains showed resistance to 35 antibiotics, particularly to tetracyclines, beta-lactams, and cephalosporins, which emphasizes the need to looking for novel options for pathogen management [86]. Similarly, a study was conducted to assess *S. iniae* antibiotic resitance present in shrimp farming. They tested amikacin, neomycin, enrofloxacin, lincomycin, and sulfamethoxazole and found that *S. iniae* exhibited resistance to these antibiotics [94]. Furthermore, the use of these antibiotics alters the intestinal microbiota of organisms, affecting beneficial microbiota. For example, a study reported that exposure to low levels of olaquindox induced alterations in the intestinal microbiota of zebrafish, leading to increased susceptibility to pathogens [95]. Meanwhile, treatments with doxycycline, oxytetracycline, and florfenicol can cause dysbiosis and metabolic disorders [96], which stresses the organism, influencing its stability and growth [97].

Microorganisms use various strategies to resist the presence of antibiotics, which include:

(1) *Alteration of the antibiotic active site*: This phenomenon is more pronounced in macrolide antibiotics and frequently observed in resistance development to beta-lactams, quinolones, and tetracyclines [98].
(2) *Enzymatic inactivation*: Most Gram-positive and Gram-negative bacteria are capable of synthesizing enzymes that degrade antibiotics. Among this group of antibiotics, beta-lactams are the most susceptible to enzymatic inactivation [99].
(3) *Reduction of inner and outer membrane permeability*: This mechanism prevents the drug from permeating into the cell [100].
(4) *Drug efflux*: The intricate bacterial machinery can expel any toxic compound that might cause potential harm. These resistance mechanisms affect a wide range of antimicrobial classes from protein synthesis inhibitors to fluoroquinolones, beta-lactams, carbapenems, and polymyxins [101].
(5) *Alternate metabolic pathways*: Another observed mechanism involves the bacterium capability to modify its metabolic pathways, causing the conventional targets of antimicrobials to disappear. An example is the acquisition of folate instead of its synthesis [102]. In addition, bacteria can transfer antibiotic resistance genes during cell division (vertical

transfer). On the other hand, genetic information can also be transferred through processes known as horizontal gene transfer (transformation, transduction, and conjugation), thus enabling the communication of new strategies to counteract the effects of antimicrobials [103].

Additionally, the excessive use of antibiotics in aquaculture can lead to another issue, which is the presence of residues in marketed fish and its derived products [96,104]. This excess can result in the accumulation of antimicrobial residues in consumers, potentially altering normal flora, increasing susceptibility to bacterial infections and posing risks of toxicity and allergenicity [105–107].

7. Strategies to combat infection

The use of antimicrobial peptides, vaccines, and peptides in combination with essential oils like clove and cinnamon are among the strategies considered to manage the spread of diseases associated with *S. iniae* [108,109].

Streptococcosis caused by *S. iniae* in aquaculture is treated with drugs such as oxytetracycline, amoxycillin, enrofloxacin, furazolidone, and erythromycin [59,110]. Notably, one of the aquaculture species that has been the focus of research is the Nile tilapia (*Oreochromis niloticus*), which is due to its significant economic impact [111] and diversification of production practices. This fish is susceptible to streptococcal infections, particularly those caused by *Streptococcus iniae* and *Streptococcus agalactiae* [112,113]. The disease is usually treated with antibiotics or vaccines, but since antibiotic-resistant strains have emerged, vaccination becomes a viable option [114,115].

In some fish farms, vaccination is routinely performed to reduce species mortality [105]. Most available vaccines are based on formulations of dead cells with formalin. Records have mentioned the use of live attenuated, recombinant, and multivalent vaccines (requiring an adjuvant) [116].

Vaccination in fish can be carried out in three ways, with one of the most popular methods being intraperitoneal injection, recommended for providing long-lasting protection [117,118]. However, this method can only be used in fish that have not yet entered the fattening stage on the farm [89]. Immersion vaccination constitutes another application method, proving beneficial for small fish [119]. Nevertheless, it cannot be utilized for larger fish due to its economic infeasibility from the producer's perspective, given the required quantity of the vaccine [120].

Another administration method is through oral delivery via the diet [120]. This option reduces application costs. However, when this approach is used, vaccines are vulnerable to the conditions within the organism gastric system [121]. Among the mentioned administration methods, oral and immersion vaccines have proved to be practical for vaccinating many fish and are easier to administer to small fish. In this regard, successful development of oral vaccines has been reported. For instance, a study reported higher levels of expression of various innate and adaptive immune genes in fish administered a bivalent vaccine composed of bacterins (formalin-killed cells) [122]. Another study demonstrated efficacy in protection against *S. iniae* in rainbow trout (*Oncorhynchus mykiss*) with an oral vaccine of alginate-chitosan enhanced by an ionotropic procedure [123]. The combination of oral and immersion application strategies has also been reported with good results [124]. However, in addition to vaccines, implementing good farm management practices and conducting effective disease diagnostics are crucial and should be considered. Moreover, vaccination should be performed well in advance of pathogen exposure, which is also essential to consider the size of the organisms and maintain control over beneficial temperature conditions [125].

To enhance the effectiveness of immunization, certain vaccines require adjuvants or immunopotentiators [126], which increase the cost of these products and, at times, render them economically unviable. These adjuvants typically have an oily base, useful for extending antigen preservation, improving retention in tissues, and enhancing their effectiveness within the organism [5,127,128].

Currently, few commercially vaccines are available, which makes it challenging to provide adequate treatment against the pathogen [129]. An approved vaccine for tilapia is AQUAVAC® Strep Si (Merck & Co., Inc., Rahway, NJ, USA) against *S. iniae*. However, its use is restricted to countries with regulatory approval [89]. Due to the limitations of existing vaccines, new approaches should address the significant diversity of *S. iniae* and provide lasting immunity [129].

Vaccines based on recombinant antigens or inactivated pathogens have been reported to have certain limitations in terms of providing long-term protection due to variations in capsular composition among strains [117,130–132]. This situation leads to the search for other alternatives, such as antibiotics or disinfectants. However, a recent study demonstrated that commercial disinfectants, such as bleach, povidone-iodine, hydrogen peroxide, Virkon® (AQUATIC), and Ovadine® (SYNDEL) do not completely eradicate the biofilm produced by *S. iniae*, recommending higher concentrations and exposure times for surfaces where the pathogen may be found [129]. Efforts have also been made to develop environmentally friendly strategies [133]. Among them strategies are plant-derived essential oils. For example,

a study demonstrated that *Cinnamomum verum* oil can be used as a preventive method against streptococcosis [4]. Another strategy is the use of probiotic bacteria, which through various mechanisms benefit host health [134]. The outcome is reflected in a reduction in disease occurrence and improved recovery from ailments [135–137].

Bacteria with probiotic potential, such as *Bacillus mojavensis* B191 and *Bacillus subtilis* MRS11 isolated from the intestinal mucosa of Nile tilapia (*Oreochromis niloticus*) have shown strong adherence to the intestinal mucosa and exhibited high competitiveness for nutrients against pathogenic microorganisms under *in vitro* conditions [138].

The synthesis of proteolytic, amylolytic, cellulolytic, and lipolytic enzymes is one of the main enzymatic effects brought on by probiotics usage and results in better food digestion [139]. Furthermore, it has been demonstrated that *Bacillus* spp. strains have the capacity to produce exoenzymes, facilitating the metabolism of various food substrates. Moreover, these strains can produce endospores, allowing them to survive adverse environmental conditions and adapt to temperature changes [140–143]. Further, *Streptococcus iniae* has been significantly inhibited by two strains of bacteria, *Bacillus mojavensis* B191 and *Bacillus subtilis* MRS11, isolated from the intestinal mucosa of Nile tilapia. After 60 days of feeding at a dose of 108 CFU/g, the same Nile tilapia demonstrated potential as an *in vivo* probiotics [144]. According to this finding, regular ingestion of the probiotic strains used in the dose might potentially have positive impacts on feeding aquaculture species. Additionally, in the presence of these probiotics, increases in production of the proinflammatory interleukins IL-1 and TNF- have been observed in both tilapia and Koi carp [145]. Moreover, the expression of anti-inflammatory enzymes (IL-10 and TGF-β) has been observed controlling the production of proinflammatory cytokines. Thereby, the gene-level effect of probiotics is demonstrate in modulating the immune system [146]. Another strain that has shown intriguing probiotic properties is *Paenibacillus ehimensis* (NPUST1) also found in the Nile tilapia [147].

Finally, recent studies have demonstrate the potential of new probiotic strains isolated from buffalo milk (*Bubalus bubalis*) and artisanal ferments, which have shown activity against both Gram-positive and Gram-negative pathogenic strains. Therefore, these strains could be exploited as antimicrobial probiotics for veterinary use [148–153].

8. Conclusions

Infections caused by *S. iniae* will persist, indirectly driven by the need to sustain and expand aquaculture operations to meet the demand for human consumption. This scenario is likely to lead to the emergence of new strains or antibiotic-resistant strains with the potential to infect humans, posing a significant challenge to public health. Despite the availability of alternatives, such as probiotics, vaccines, and antimicrobial peptides to prevent infection in farmed fish, the continued use of antibiotics persists due to the inefficacy of the aforementioned strategies, attributable to the inherent variability of the strains.

Therefore, the quest for infection control strategies remains a challenge. Nevertheless, the importance of enhancing and optimizing pathogen identification methods is underscore to facilitate accurate diagnosis and the appropriate application of specific treatments.

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