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Research Article

Bacterial pathogens identification in farmed barramundi (*Lates calcarifer*) at Bushehr and Hormozgan province, Iran

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Abstract

The Asian sea bass (*Lates calcarifer*), also known as barramundi, is a species of great importance in Iran's aquaculture industry. This fish has been farmed for many years to fulfill the rising domestic demand for fresh seafood and to promote exports. However, bacterial diseases and infections pose a significant threat to the growth of this popular species, leading to substantial losses. This study aimed to investigate bacterial contamination in barramundi at coastal cage farms located in the Bushehr and Hormozgan provinces of Iran, as well as to identify the signs and lesions resulting from these infections. According to the criteria for bacterial infection, 30 fish samples were collected from farms. Necropsies were performed on the collected fish, followed by bacteriological analysis and genotyping using 16S rRNA sequencing. The bacteriological results revealed five bacterial strains, confirmed through agent isolation, biochemical profiling, and 16S rRNA sequence verification. The identified isolates comprised *Streptococcus iniae* strain SB1 (60%), *Streptococcus agalactiae* strain 20 (70%), *Shewanella algae* strain 16 (60%), and *Vibrio* species such as *V. azureus* strain N3 (86%) and *V. harveyi* strain N4 (90%). The identification of bacterial species is crucial for resolving health issues and reducing mortality in farmed barramundi. This study comprehensively identified bacterial pathogens impacting barramundi in Iran. The findings, along with the detection methods and bacterial genotyping techniques used, offer practical insights for future research in this area. Additionally, this study increases awareness among researchers, farmers, and clinicians, facilitating the adoption of effective management strategies in aquaculture practices.

Introduction

The global demand for fish is rising rapidly, resulting in wild fisheries being either fully exploited or overexploited (Oujifard *et al.*, 2014; Ndashe *et al.*, 2023). As a result, the aquaculture industry is growing rapidly worldwide to address this issue (Sansuwan *et al.*, 2023). In this context, Iran, with its extensive 5,800 km coastline (including islands), possesses significant potential for utilizing water resources and cultivating marine aquatic species in this specialized field (Harlioglu and Farhadi, 2017). This distinct advantage has facilitated the introduction of several new species, such as barramundi or sea bass (*Lates calcarifer*), which are well-suited for human consumption and support-related business ventures (Oujifard *et al.*, 2014; Jena *et al.*, 2019; Chew and Gibson-Kueh, 2023).

The *Latidae* family includes the genera *Lates* and *Latilus*, commonly referred to as barramundi (Jena *et al.*, 2019; Vo *et al.*, 2020). Barramundi is distributed across various regions, from the western Indian Ocean to the Pacific Ocean, including areas such as the Persian Gulf, China, Taiwan, Papua New Guinea, and northern Australia. They are euryhaline, displaying a strong tolerance for different salinity levels. Barramundi are classified as catadromous fish, migrating from freshwater and brackish environments to saltwater during the breeding season (Jerry, 2014). In Iran's southern provinces, barramundi are breded mainly in earthen ponds and coastal cages. The fish market demand, favorable reproductive traits, and the suitable climate in south Iran have heightened farmers' interest in farming (Oujifard *et al.*, 2014).

Barramundi, though highly suitable for aquaculture, are particularly susceptible to bacterial infections, with Vibrio species being a major concern (Ajdari, 2018). Key bacterial pathogens affecting barramundi include *Vibrio harveyi*, *Streptococcus iniae*, and *Streptococcus agalactiae*. Among these, *S. iniae* poses a significant threat to Australian aquaculture, with strains causing mortality rates as high as 60% to 80%. Infected fish display a range of clinical signs, including unilateral or bilateral exophthalmia, corneal opacity, hemorrhaging at the fin base, skin discoloration, tail rot, and erratic swimming behavior (Erfanmanesh *et al.*, 2019; Izwar *et al*., 2020; Awate *et al*., 2023).

However, there is a considerable gap in comprehensive foundational knowledge about bacterial contamination, diseases, clinical signs, and postmortem lesions linked to bacterial infections in barramundi farming within coastal cages in Iran and other Persian Gulf countries (Erfanmanesh *et al*., 2019). Current data on bacterial pathogens affecting barramundi are limited, frequently concentrating on individual pathogens or specific farms, with some studies offering conflicting results. To bridge this gap, the present study aimed to accurately identify bacterial contaminations associated with barramundi farming in floating cages across two southern provinces of Iran while also gaining a deeper understanding of the clinical signs and necropsy findings related to these pathogens. The findings of this study will offer valuable insights and prognoses regarding the types and prevalence of bacteria that may be present in barramundi farms.

Materials and methods

Sampling sites

In the Iranian provinces of Bushehr and Hormozgan, three barramundi breeding sites along the northern shores of the Persian Gulf were selected for sampling (Fig. 1). The fish were raised in coastal cage sites covering an area of 1,000 m², with a water depth of 1.5 meters. These ponds were supplied with recirculating seawater, maintaining a salinity of 40 ppt and a dissolved oxygen level of 13 mg/L. The fish density within the ponds was kept at 40 kg/m^2.

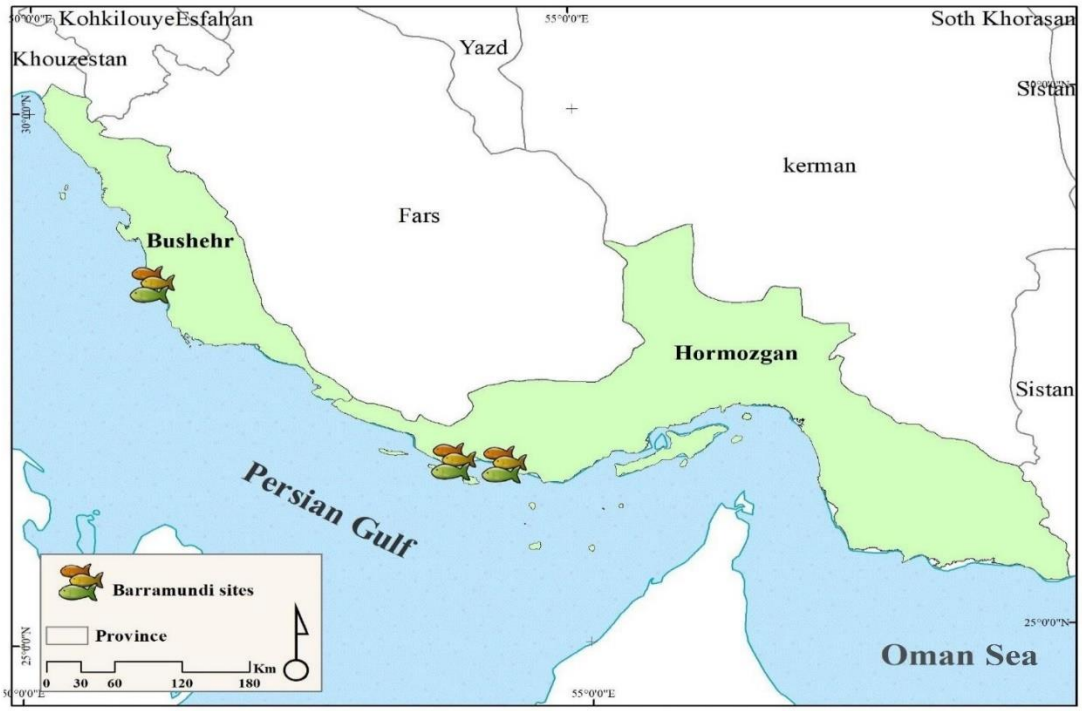

Figure 1: Map showing the locations of three barramundi coastal cage sites in the Bushehr and Hormozgan provinces.

Sampling and necropsy

Ten fish exhibiting abnormal signs, such as body darkening, exophthalmia, erratic swimming, ulcerative lesions on the operculum and around the mouth, and abdominal swelling, were selected from each cage. The selected fish ranged in weight from 30 to 350 grams and in length from 11 to 45 cm. They were transferred to a clean, covered area and euthanized with an overdose of Tricaine mesylate (MS-222) (0.1%, Sigma Chemical Co., USA). After euthanasia, their body surfaces were disinfected with 70% alcohol, and a necropsy was performed. Samples were taken from the gills, skin lesions, and internal organs, including the liver, kidney, spleen, and brain, for microbiological analysis and pathogen isolation. Each fish was thoroughly examined for visible signs of gross lesions. The collected samples were then sent to the Microbiology Laboratory at the Faculty of Veterinary Medicine, University of Tehran, Iran, under controlled conditions for further analysis.

Identification of bacterial strains

To obtain pure bacterial strains following standard methods (Quinn, 1994, Markey *et al.*, 2013, Buller, 2014), samples were inoculated onto various agar media, including Tryptic Soy Agar (TSA), Muller Hinton Agar (MH) supplemented with 1.5% NaCl, and Blood Agar. The inoculated plates were incubated at 24°C for 2 to 5 days. Colonies with distinct visual characteristics were subcultured from single colonies to ensure purity. Pure cultures were then maintained on Muller Hinton Agar (with 1.5% NaCl) at 25°C. The macroscopic features and appearance of the purified colonies, including size, consistency, color, and other attributes, were carefully assessed. The bacteria's morphology was observed under a light microscope after performing Gram staining.

Biochemical tests and genotype determination were carried out in accordance with standard protocols. The biochemical tests were performed using 24-hour bacterial cultures, with the results assessed after 48 hours of incubation at 25°C. To ensure precision and reliability, each experiment was repeated three times. A variety of biochemical assays were conducted, including tests for oxidase, catalase, hemolysis, oxidation/fermentation, nitrite/nitrate reduction, indole production, esculin hydrolysis, growth at different temperatures (10°C and 42°C), growth in the presence of 6.5% NaCl, arginine hydrolysis, Kligler's iron agar (KIA) test, lysine and ornithine decarboxylase, Methyl Red/Voges-Proskauer (MR/VP) test, Simmons citrate utilization, colony features on TCBS, motility, hydrogen sulfide production, beta-galactosidase activity, gelatin and urease hydrolysis, DNase activity, CAMP test (for Gram-positive cocci), and lipase activity.

The biochemical characterization of each strain was performed following the protocols outlined in Clinical Veterinary Microbiology E-Book (Markey et al., 2013) and in accordance with the criteria set forth by Bryant *et al*. (Bryant *et al.*, 1986) and Austin (Austin, 2011; Varalakshmi *et al.*, 2022). These well-established guidelines and criteria were instrumental in accurately identifying and characterizing the bacterial strains based on their biochemical traits and phenotypic characteristics.

16S rRNA sequence analysis

The axenic cultures of each isolate underwent genotyping via 16S rRNA analysis. DNA extraction was carried out using the DNeasy Blood and Tissue kit (Qiagen). The quality and concentration of the extracted DNA were evaluated by measuring absorbance at 260 nm and 280 nm. For the analysis, the primers 16S-27f (5'- GAGTTTGATCCTGGCTCAG -3') and 16S-1505r (5'- GATACGGCTACCTTGTTTACGA -3') were used.

The PCR reaction mixture, with a total volume of 50 µL, included a reaction buffer containing 1.5 mM MgCl2, 50 mM KCl, and 10 mM Tris-HCl at pH 8.3. It also contained 100 µM of each deoxynucleoside triphosphate (Promega, Madison, WI, USA), 0.2 µM of each primer, and 1.5 U of Taq polymerase (Sigma, St. Louis, MO, USA). The PCR amplification process involved an initial denaturation step at 95°C

for 3 minutes, followed by 35 cycles of amplification, each consisting of 45 seconds at 93°C, 60 seconds at 58°C, and 90 seconds at 72°C. The resulting PCR amplicons from each isolate were purified using a GF-1 PCR Clean-up kit and visualized by electrophoresis on a 2% agarose gel to confirm the purified products.

The nucleotide sequences of the purified PCR products were analyzed using an ABI 3730XL DNA Analyzer with automated Sanger dideoxy fluorescent nucleotide sequencing. These sequences were subsequently submitted to the GenBank database for registration. To identify closely related bacteria, the sequences were compared with the extensive sequence-based taxonomic data available in the GenBank database via the NCBI BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple alignments of the 16S rDNA sequences (approximately 1.45 kb in length) with closely related taxa were performed using a maximum likelihood model to generate a phylogenetic tree. The neighbor-joining method, implemented with MEGA version 11 software, was used to construct the tree, and a bootstrap analysis with 1000 replicates was conducted to evaluate its robustness.

Results

From the 30 barramundi samples, five bacterial isolates were obtained and identified. These isolates were as follows: *S. iniae* strain SB1 (GenBank accession no. MG912577.1), *S. agalactiae* strain 20 (GenBank accession no. MG597066.1), *Shewanella algae* strain 16 (GenBank accession no. MG597061.2), *Vibrio azureus* strain N3 (GenBank accession no. MN367963.1), and *V. harveyi* strain N4 (GenBank accession no. MN382341.1). The bacteriological identification was consistent with established references, validating the identification process (Quinn *et al*., 2011; Markey *et al*., 2013; Buller, 2014). Sequence analysis revealed that the amplified products from these isolates closely matched reference strains available in the GenBank database, further confirming the accuracy of the bacterial identification (Sayers *et al*., 2024) (Table 1).

The necropsy findings further confirmed the presence of bacterial infection in the sampled fish. Out of the 30 barramundi examined, 24 (80%) displayed various signs of infection. These signs included exophthalmia (Popeye), body darkening, ulcerated lesions, hemorrhaging around the operculum or mouth, severe damage to internal organs, abdominal swelling, ascites (fluid accumulation in the abdominal cavity), and nodules in the kidney and spleen. Externally, the fish showed exophthalmia, darkened bodies, and ulcerative lesions, characterized by red, swollen, or raised areas on the skin, fins, or mouth. Hemorrhaging was noted around the operculum or mouth, along with abdominal swelling and ascites. Internally, the fish had severely damaged viscera, with signs of tissue necrosis, and nodules were present in the kidney and spleen.

The isolation frequency for *V. harveyi*, *V. azureus*, *S. agalactiae*, *S. algae*, and *S. iniae* were 90%, 86%, 70%, 60%, and 60%, respectively. In terms of organ-specific bacterial isolation, *S. iniae* was found in the

kidney (12 cases, 40%) and brain (6 cases, 20%), while *S. agalactiae* was isolated from the kidney (8 cases, 26.7%). *S. algae* was primarily isolated from skin lesions (18 cases, 60%). *V. azureus* was recovered from the kidney (14 cases, 46.7%), spleen (3 cases, 13.4%), and skin lesions (11 cases, 50%). Similarly, *V. harveyi* was isolated from the kidney (13 cases, 53.4%), spleen (4 cases, 16.7%), and skin lesions (10 cases, 40%). No bacterial isolates were recovered from the gills or liver.

Table 1: Biochemical analysis of the five bacterial isolates.

Characteristic	*S. iniae*	*S. agalactiae*	*V. harveyi*	*V. azureus*	*S. algae*
Gram reaction	+	+	-	-	-
Cell morphology	Cocci	Cocci	Curved-Rod	Curved-Rod	Rod
Oxidase	-	-	+	+	+
Catalase	-	-	+	+	+
Motility	-	-	+*	+*	+*
Methyl Red (MR)	NT	NT	+*	-*	+*
Voges Proskauer (VP)	-	+	+ (48h)*	+ (48h)*	-*
Hemolysis (5% sheep RBC)	β	-	α/β	-	-
Citrate utilization	-	-	+	+	-
Urease	-	-	-	-	-
Nitrate reduction	-	-	+	+	+
Gelatinase	-	-	+	+	+
CAMP	+	-	NT	NT	NT
Indole	-	-	+*	+ (48h)*	+ (48h)
O/F	F	F	F	F	F
Hydrogen sulfide (H_2S)	-	-	-	-	-
ONPG	-	-	-	-	+ (48h)
Arginine dihydrolase	-	-	-*	-*	-*
Lysine decarboxylase	NT	NT	+*	-*	+*
Ornithine decarboxylase	NT	NT	-*	-*	+*
Grow on/in:					
MacConkey Agar	-	-	+	+	+
Kligler's Iron Agar	NT	NT	ALK/Acid	ALK/Acid	ALK/Acid
TCBS	NT	NT	Yellow (1.5 mm)	Yellow (1.5 mm)	Yellow (2 mm)
Hippurate	-	+	NT	NT	NT
Esculin	+	-	+	+	+
Mannitol	+	-	+	+	+
NaCl 6.5%	-	-	+	+	+
2, 4, 6, and 8 % NaCl	NT	NT	+	+	+

NT = Not tested, + = Positive, - = Negative, * = 1% NaCl has been added to the medium.

Of the six fish (20%) that showed no visible lesions indicating bacterial infection, *S. iniae* was the only bacterium isolated from kidney samples. However, bacterial co-infections were detected in 14 cases (46.7%). Among these co-infections, *S. iniae* and *S. agalactiae* were each found in 11 cases (36.7%). Additionally, *S. iniae*, *V. harveyi*, and *S. algae* co-infections occurred in 3 cases (10%) each. All 11 fish with co-infections displayed signs consistent with concurrent bacterial infections.

The 16S rRNA sequence analysis revealed that the amplified 1458 bp product from *S. iniae* showed a 99.86% similarity with the reference strain *S. iniae* ATCC 29178 (GenBank accession no. DQ303187). Similarly, a 1486 bp fragment from *S. agalactiae* demonstrated 100% similarity with the reference strain *S. agalactiae* ATCC 13813 (GenBank accession no. AEQQ01000116). Both *V. harveyi* and *S. algae* produced 1476 bp fragments, with *V. harveyi* showing 99.66% similarity to the reference strain ATCC 35084 (GenBank accession no. EU130475.1) and *S. algae* exhibiting 100% similarity to ATCC 51192 (GenBank accession no. NR_117771). For *V. azureus*, the 1468 bp fragment displayed 99.66% similarity to the reference strain *V. azureus* NBRC 104587 (GenBank accession no. BATL01000140). To further confirm species identification, a phylogenetic tree was constructed using the 16S rRNA sequences of all the bacterial isolates from this study, along with type/reference strains and their closest taxa retrieved from the GenBank database. The phylogenetic analysis validated the accurate identification of all five bacterial isolates (Fig. 2).

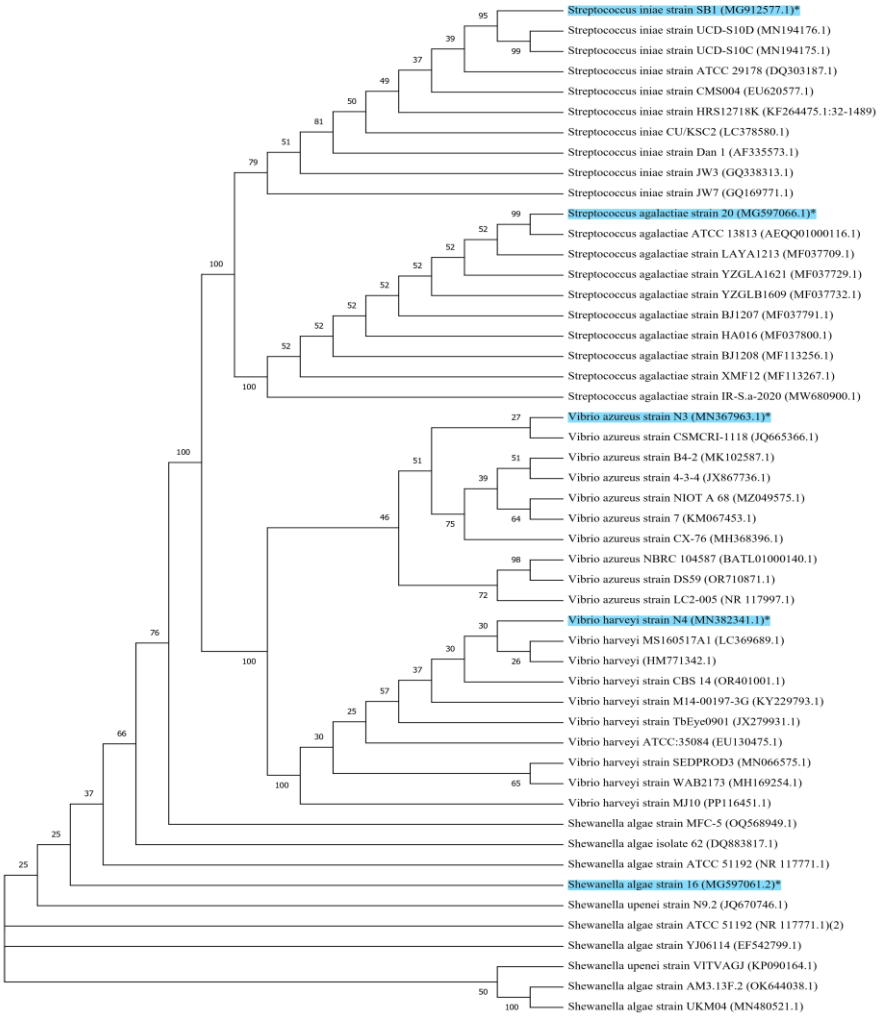

Figure 2: A phylogenetic tree (maximum likelihood) constructed using 16S rRNA gene sequences of all bacterial isolates. The strains identified in this study are highlighted and marked in blue.

Discussion

Given the rapid expansion of marine fish farming in floating cage systems in southern Iran, it is essential to explore the factors contributing to disease outbreaks in these conditions. Bacterial diseases, in particular, present a significant challenge to the growth of aquaculture, as seen in many leading countries in this industry. This issue serves as a warning to farmers and fisheries planners in these regions, emphasizing the need for proactive measures to combat these challenges (Dong *et al*., 2017; Hooshmand *et al*., 2022). The current study focused on identifying bacterial pathogens that may be responsible for body lesions and mortality in barramundi in southern Iran. This research provides a comprehensive report on bacterial pathogens affecting barramundi in the region, offering valuable insights for developing management strategies to prevent bacterial infections.

The findings from the present study are highly valuable for fish farmers, providing essential guidance on both treatment and prevention strategies. Monitoring bacterial pathogens like *S. iniae*, *S. agalactiae*, *Vibrio* spp., and *S. algae* is critical, as these organisms can lead to severe diseases and significant economic losses. A lack of awareness about the types and levels of bacteria present in an aquaculture system increases the risk of widespread bacterial outbreaks (Talpur, 2014; Yern *et al*., 2022). Introducing vaccination programs offers a cost-effective approach to preventing infectious diseases and promoting long-term sustainability in the aquaculture industry (Ahangarzadeh *et al*., 2023).

The isolation of bacterial pathogens from observed lesions highlights the vulnerability of barramundi in Bushehr and Hormozgan provinces, stressing the need for preventive measures. The presence of these pathogens may be linked to stressful rearing conditions, such as poor water quality, high ammonia levels, overcrowding, malnutrition, nutrient deficiencies, transportation, and handling (Fotedar, 2016). While previous studies have investigated pathogens associated with these bacteria, they often focused on a single agent or induced experimental infections to evaluate vaccine immunogenicity (Erfanmanesh *et al*., 2019; Ahangarzadeh *et al*., 2023). As a result, comparing bacterial species and levels with studies that isolate multiple bacterial species in Asian seabass farming proves challenging. In Hooshmand *et al*. (2022) study, *V. alginolyticus* was isolated from various fish species in the south of Iran and they reported clinical signs and bacterial isolation methods similar to those observed in this study.

The pathogenicity of the bacteria isolated in the current investigation for barramundi has been highlighted in several studies (Ahangarzadeh *et al*., 2023). Bacterial species such as *Streptococcus*, *Vibrio*, and *Flexibacter* are known to be significant pathogens in barramundi. These bacteria raise concerns about potential contamination in barramundi and the associated risks to consumers. Although transmission of these bacterial agents to humans is rare, research has shown that bacteria are the most frequent cause of disease transmission from fish or fish farms

to humans (Austin, 2010; Rahman *et al*., 2020).

In a separate study conducted in 2018, 110 barramundi from farms in the southern provinces of Iran (Khuzestan, Bushehr, and Hormozgan) were tested for the presence of *V. harveyi*. Of the 95 bacterial isolates identified, 65 were classified as *Vibrio* species, with 46 specifically identified as *V. harveyi*. These findings indicated a high prevalence of *V. harveyi* in Asian sea bass at the farms studied. The sampling procedures, bacterial identification techniques, and characteristics of *Vibrio* species observed in this study were comparable to those in the present research. However, this study lacked genomic and phylogenetic analyses (Ajdari, 2018).

Vibrio poses a remarkable annual threat to the economic viability of barramundi farming in floating cages. Vibriosis, a highly destructive disease in marine environments, impacts various fish species, including barramundi. Pathogenic *Vibrio* species in marine fish are particularly concerning, as they cause systemic infections that can lead to fish mortality and may even present a risk of disease transmission to humans (Hooshmand *et al*., 2022). In a study by Ransangan and Mustafa, 21 *Vibrio* isolates from diseased barramundi were analyzed. Initially, conventional biochemical methods identified four isolates as *V. harveyi*, 16 as *V. parahaemolyticus*, and one as *V. alginolyticus*. However, 16S rRNA sequencing revealed a genetic similarity of 98% to 100% among all isolates, ultimately classifying them as *V. harveyi* (Ransangan and Mustafa, 2009). This underscores the limitations of relying solely on conventional biochemical methods for identifying *Vibrio* species. Hooshmand *et al*. emphasized the critical role of molecular identification techniques (Hooshmand *et al*., 2022). The use of molecular methods in the present study provided greater accuracy in pathogen identification, and registering these strains in databases will support future research efforts in this field.

In the aquaculture industry, *Vibrio* species are significant opportunistic pathogens for marine fish, causing the disease vibriosis. These bacteria are commonly found in the gills, skin, intestines, and other internal organs of infected fish. However, *Vibrio* can also naturally occur on the skin and in the digestive system of healthy fish, suggesting that the presence of *Vibrio* does not necessarily indicate infection. Therefore, a definitive diagnosis requires isolating the bacteria from the kidney and other organs showing lesions (Noga, 2010; Hooshmand *et al*., 2022). In this study, *Vibrio* species were isolated from various organs of diseased fish, including the kidney, skin lesions, and spleen, specifically targeting these organs to assess the presence and distribution of *Vibrio* bacteria in the affected fish.

Streptococcosis, caused by *S. iniae*, is a severe infection impacting both marine and freshwater environments, often leading to high mortality rates. The disease presents in two clinical forms: subacute and acute. In the subacute form, fish typically exhibit signs such as exophthalmia, skin darkening, and erratic swimming behavior (Shinn *et al*., 2023). These signs were also observed in the fish sampled for the current study. Similarly, a 2023 study by Awate *et al*. on

Asian seabass infected with *S. iniae* in Singapore and Australia reported clinical signs consistent with those found in the present study (AwateMubarka and Huber, 2023).

Streptococcosis accounts for approximately 1% of fish mortality (Van Doan *et al*., 2022). In the subacute form, few clinical signs may be evident, with exophthalmia being the most common. This form can be particularly destructive, causing significant losses, especially during nighttime. In Australia, barramundi farmed in sea cages have faced annual losses ranging from 8% to 15% due to *S. iniae*, with outbreaks sometimes leading to losses as high as 70%. Research has indicated that wild fish near seabass breeding cages act as a reservoir for *S. iniae* (Bromage and Owens, 2002; Van Doan *et al*., 2022), underscoring the need for management strategies to prevent wild fish from entering the cages. Lowering stocking density has also been shown to improve disease control (Van Doan *et al*., 2022). Additionally, *S. iniae* is recognized as a zoonotic agent, posing a risk to humans who come into contact with infected fish, potentially leading to cellulitis in the hands or even endocarditis (Delannoy *et al*., 2013).

Vibriosis, a fatal hemorrhagic septicemia, is caused by *Vibrio* species. In the Philippines, non-cage-reared barramundi experience a 2-3% mortality rate from *Vibrio* infections, particularly after heavy rainfall (Zarei *et al*., 2021; Rahman *et al*., 2022). *Vibrio* spp. are common in many marine, freshwater, and river fish species, often linked to poor water quality, stress, malnutrition, and parasitic infections. Infected fish typically exhibit extensive systemic and skin hemorrhaging, skin wounds, exophthalmia, and red belly (Krupesha Sharma *et al*., 2012). Necropsies often reveal necrosis and bleeding in the kidney, liver, and spleen. Due to the severe impact of this disease, considerable research has focused on the virulence factors of *Vibrio* spp., along with the development of rapid diagnostic methods and preventive strategies (Frans *et al*., 2011; Mohajeri *et al*., 2011; Xu *et al*., 2021).

Infectious diseases cause substantial economic losses in aquaculture, but many can be prevented through the implementation of biosecurity protocols. Key biosecurity measures include health inspections, quarantine and treatment, egg disinfection, maintaining hygiene for equipment, regulating human traffic, treating inlet water, implementing wastewater treatment, using clean feed, and ensuring proper waste disposal. Additionally, minimizing interactions between wild and farmed fish is critical. Disease outbreaks often occur when pathogens spread within a unit and become challenging to eliminate, especially when the causative agents are unknown. In such cases, vaccination and drug treatments are employed to reduce infection rates and limit losses. Adhering to biosecurity principles is the most effective strategy for disease prevention. These considerations underscore the importance of studies like the present one in key breeding regions to tackle these challenges (Jerry, 2014).

Many studies have explored the interactions between fish, bacteria, and diseases, highlighting their crucial role in fish pathology (Johnson and Paull, 2011).

The local climatic conditions of a specific area, region, or country greatly influence the occurrence and development of bacterial diseases in fish. Consequently, fish farmed in regions like the Persian Gulf in Asia, the Mediterranean Sea in Europe, and Northern European countries face distinct health challenges. It is essential to recognize that findings from studies conducted in one region may not be directly applicable to another. This underscores the importance of region-specific research, such as the present study, to address the unique conditions and challenges of each location (Pękala-Safińska, 2018; Zaheen *et al*., 2022).

Conclusions

Barramundi is a promising species for cultivation in Iran's sea cages and earthen pond systems, valued in both domestic and international markets for its rapid growth and adaptability to diverse environmental conditions (Oujifard *et al*., 2014). Investigating the pathogenic microorganisms associated with barramundi farming not only raises awareness and aids in disease prevention but also enhances production efficiency and marketability. This study identified and introduced bacterial species responsible for internal and external lesions, as well as disease development. The findings, along with the bacterial detection and genotyping methods used, provide valuable insights for future research, offering practical applications for researchers, breeders, and clinicians in this field. Additionally, these results can support the development of effective management strategies for breeding farms.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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