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Study On Isolation and Identification of Bacteria from Infected Fish (*Channa Striata*)

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Abstract

The current research was aimed at isolation and characterization of disease-causing bacteria from infected *Channa striata* (Murrel). Infected fish specimens with visible symptoms like haemorrhagic lesions, fin erosion, and abdominal distension were retrieved from aquaculture fishponds. Routine bacteriological techniques were employed, namely, isolation on nutrient and selective agar medium, Gram staining, and biochemical characterization for bacterial identification. The Gram staining test indicated the occurrence of both Gram-positive and Gram-negative bacteria, suggesting polymicrobial infection. Most isolated bacterial genera of frequent occurrence were Bacillus, Streptococcus, Aeromonas, Pseudomonas, and Vibrio, which are recognized fish pathogens that induce septicemia, gill rot, and ulcerative lesions in freshwater fishes. The results demonstrate the importance of systematic bacterial isolation and identification in disease aetiology research. Such research is valuable for the preparation of preventive health protocols, proper diagnosis, and treatment regimens to reduce economic loss in Murrel culture.

Keywords: Channa striata, Bacillus, Streptococcus, Gram staining, bacterial pathogens, fish disease, aquaculture management.

1. Introduction

Aquaculture is currently among the world's most rapidly growing industries that produce food and is a critical component in the fulfilment of the increasing global demand for animal protein (Gufe et al., 2019). In addition to providing food, it also generates economic growth and livelihoods for millions of people (Muhammad et al., 2020), particularly in developing nations where freshwater fish culture is a significant livelihood activity. It is also driven by dwindling wild fish stocks, rising world population, and altered dietary trends that lead to heightened consumption of fish. Freshwater species are especially in demand since they have high growth rates, adaptability to varied habitats, and richness in nutrients—thus ranking high in local food and export markets. Given that it is say very inexpensive when compared to other meat products like beef and chicken most people can buy it (Tacon & Metian, 2013).As aquaculture becomes more intense, the vulnerability to disease outbreaks also grows. Among all the challenges that fish farmers experience, diseases, especially bacterial disease, are the worst. These diseases can rapidly develop, kill thousands of fish simultaneously, and even poses some danger to human lives (Mhlanga et al., 2010). Additionally, it involves other smaller organisms like the aquatic-native Vibrio genus which process a

major health risk to people when consumed with raw or improperly prepared seafood (Muhammad et al., 2020)). Diseased fish may have open sores on their bodies, ulcers, bleeding, damaged gills, or even rotting internal organs (Dutta et al., 2014). This not only damages the health and welfare of the fish but also undervalues them in the market, in some instances causing destruction of the entire stock (Shaik sana sulthana et al, 2024,).

A few bacteria infect freshwater fish, some of which include Aeromonas hydrophila, Pseudomonas species, Escherichia, Klebsiella, and Salmonella are a few bacterial genera that have been isolated from fish and may sign of multisource pollution (Sichone et al., 2013; Sichone et al., 2014)). These bacteria are "opportunistic," i.e., they infect when fish are stressed or debilitated—due to unsatisfactory water quality, overcrowding, sudden water temperature fluctuation, or inadequate diet (Rastogi, 2007).

Having such diseases begins with being precisely certain which pathogen is the problem. Conventionally, this means isolating bacteria from sick fish, cultivating them on culture media, and examining their appearance under a microscope, e.g., by Gram staining. These old reliable techniques are still heavily used because they're cheap and suitable for routine farm diagnostics. More sophisticated methods such as biochemical profiling, PCR, and 16S rRNA gene sequencing can more precisely identify and even detect new emerging pathogens. Although these aren't yet in every fish farm or laboratory, applying them to future studies may dramatically increase the way that we monitor and control bacterial disease.

Fish disease is not all about economics humans can also be impacted. Some bacteria on fish, such as Aeromonas and Streptococcus, can infect human beings on exposure to infected fish, infected water, or raw seafood consumption. This is higher for people with open wounds, immune compromised patients, or certain medical conditions. According to the current data, microbial contaminants are one of the main reasons why people get sick from eating certain foods (Matthews et al., 2017). The issue that motivated this research was the high microbial load found in fish skin, gut, or gills, the issue of food safety posed by microorganisms, as well as aquaculture’s great susceptibility to contamination from home, industrial, and agriculture waste (Muhammad et al., 2020).

MATERIALS AND METHODS

Study Area

- The present study was carried out in Siddipet, Telangana, India, focusing on the Siddipet Integrated Fish Market, which serves as a major hub for freshwater fish trade in the region.
- The market was selected as the study site due to its high fish density, Poor sanitary conditions, and increased risk of bacterial infections.
- The target species, *Channa striata* (Murrel)was chosen for its economic importance, high Nutritional value, and strong consumer demand in Telangana.

Identification of Species

Murrel fish was chosen for this project from the local integrated fish market (Siddipet) because it is widely eaten and valued for its good taste and nutritional quality.

Fig 1: *Channa striata* (Murrel)

CLASSIFICATION

- Kingdom: Animalia
- Phylum: Chordata
- Class: Actinopterygii
- Order: Anabantiformes
- Family: Chanidae
- Genus: Channa
- Species: *Channa striata*

Channa striata is defined by its long cylindrical body, wide head, large mouth with pointed teeth, and greenish to dark brown colour with irregular patches of bands on the body. *Channa striata* is an air-breathing fish that can tolerate low-oxygen levels and is therefore adaptative in the Telangana seasonal water bodies. *Channa striata's* nutritional richness has spurred its growing demand in Telangana fish markets. Apart from nutrition, its culture and commerce offer employment, upholding the livelihood of thousands of fishermen and vendors. Due to its highly prized and economically significant status, it is of utmost significance to protect this species from bacterial contamination.

1.Source of Sample

- The initial step of microbial work is the procurement of good-quality biological samples since the quality and status of the samples determine the reliability of the results.
- Fish samples were procured from the Siddipet Integrated Fish Market, within a few hours of harvest. Fresh Murrel (*Channa striata*) with visible signs of infection—ulcer, body lesions, and fin rot—were selected, suggestive of possible bacterial infection.

2. Isolation of Bacteria

Step 1: Sample Collection by Swabbing from Infected Site

- Ulcerated external body parts mucus was swabbed in light with sterile cotton swabs under aseptic conditions to obtain only the target bacteria and rule out contamination.
- Then dissolve the swabbed cotton in distilled water and make the sample.

Note: Distilled water is preferred to avoid any interference from salts or minerals that may affect bacteria viability.

Step 2: Serial Dilution

Fig 2: Serial Dilution

- Seven clean test tubes each containing 9 ml of distilled water were taken.
- 1-millilitre of fish homogenate was filled in the first test tube and shaken.
- Then, step by step, 1 ml was added from tube to tube by using sterile pipette to obtain dilutions of 10^{-1} to 10^{-7}.

(Serial dilution is done to reduce microbial concentration for easier counting and isolation of colonies.)

Step 3: **Nutrient Agar Preparation**

A mixture of 5.6 g of nutrient agar powder and 1 g agar-agar were dissolved in 250 ml distilled water in a conical flask

- Heat this mixture while stirring to fully dissolve all components on a heating mantle until dissolved.
- Autoclaved in an autoclave at 121 °C for 15 minutes.
- Petri plates were also autoclaved as a sterilization process.

Fig 3: Autoclaving the Petri plates & nutrient agar

- Once the Nutrient agar has been autoclaved, allow it cool but not solidify.
- After the medium was cooled to 45–50 °C.
- Pour nutrient agar into each plate by pour plate method and leave plates on the sterile surface until the agar has solidified.


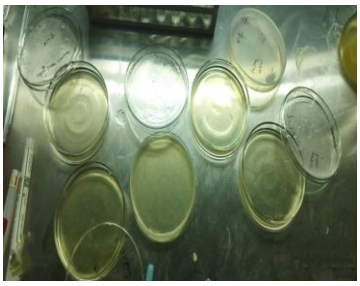

Fig 4&5: Pour plating method & Solidifying the Agar plates

Step 4:

Seven serial dilution samples were spread on to seven replicate nutrient agar plates using a 0.1 ml micropipette.


Fig 6&7: Adding sample to agar plates by using micropipette


Fig 8&9: spreading on plates with spreader & incubating the plates

Then spread on the plate with a spreader, allowed to set, and incubate the plates in incubator at 37°C for 24 hours.

Step 5: **Mother culture colony observation and selection**

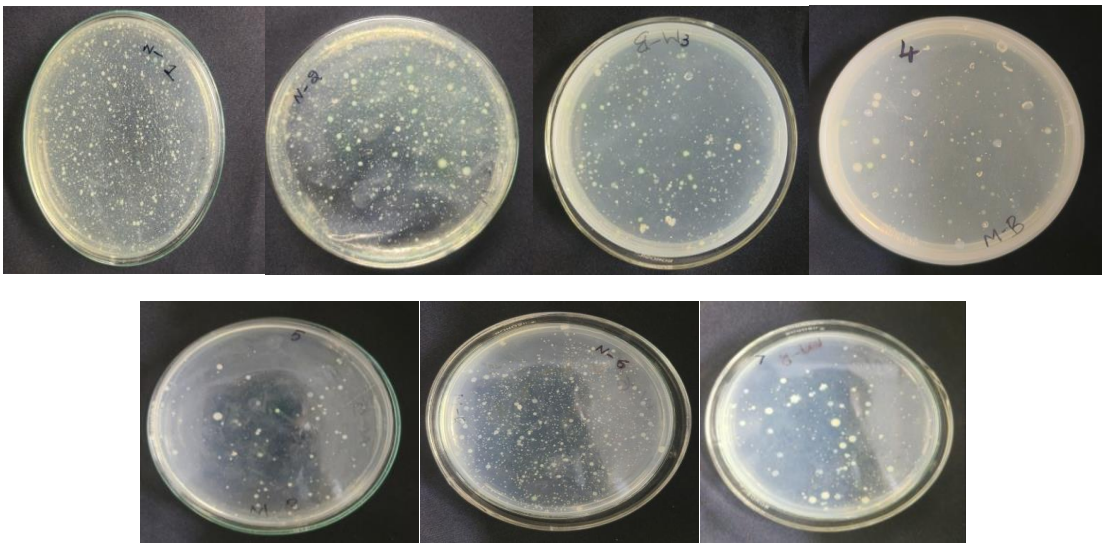

Fig 10: Observation of mother culture colony and selection

After 24hrs of incubation. Check incubated plates for colony morphology (size, shape, and colour) and select single colonies on a sterile inoculation loop.

Step 6: Pure culture colony streaking and picking

Streak-pick colonies on new agar plates by quadrant streaking to isolate pure cultures to use in further tests.

Step 7: After streaking, incubate the plates under the same conditions for 24 hours.

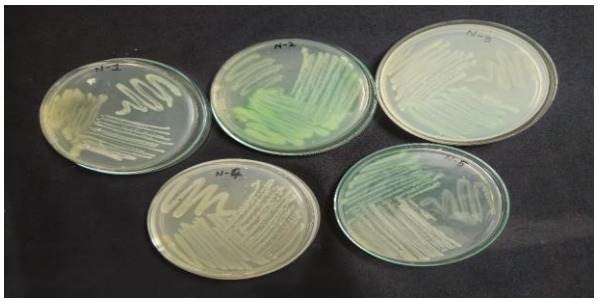

Fig 11: Pure culture

3.Bacteria Identification

Gram staining

- Gram staining is the initial and most elementary technique of bacterial identification, to classify bacteria into:
- Gram-positive (Purple): Possess a heavy peptidoglycan layer in their cell wall, retaining the stain crystal violet.

Gram-negative (Pink/Red): Possess a light peptidoglycan layer and outer membrane, losing the crystal violet and safranin colour.

Gram staining is differential staining in which bacteria are separated into Gram-positive and Gram-negative based on cell wall composition.

Fig12: Gram staining reagents

Procedure:

1. Pure culture – Take pure culture after 24 hours incubation. Take isolated colonies from the agar plate in a sterile loop.

2. Prepare smear – Place colony on a clean slide, spread thin smear, air-dry, and heat-fix or methanol-fix.

Step 1 - Crystal violet – Flood slide, briefly rinse with distilled water.
Step 2 -Gram's iodine – Flood, rinse with distilled water.
Step 3 -Decolorize (95% Ethanol) – Until runoff is nearly clear, rinse immediately with distilled water.
Step 4 -Safranin – Flood, rinse with distilled water, blot dry.

4. Microscopy Observation

Next Observe the slide under a compound microscope

- Use the oil immersion objective (100×)
- Gram-positive → purple
- Gram-negative → pink

Result

Plating and isolation of the bacteria from infected *Channa striata* (Murrel) provided robust microbiological evidence. Infected fish samples taken from the Integrated Fish Market showed classical signs of bacterial infection, such as ulcerated skin lesions, haemorrhagic spots, and scaling of skin. These are general signs of severe bacterial invasion and virulent tissue damage in freshwater fish. When the tissues of the lesion were cultured on Nutrient Agar, normal bacterial growth was observed between 24–48 hours of incubation at 28–30°C. Well-defined colonies were observed to be predominantly round in shape with white to creamy colour and smooth margin. Nutrient Agar proved to be an appropriate general-purpose medium for the culture of various species of bacteria found in the infected tissues. The sizes of colonies range from tiny pinheads to some that are spherical and uneven and spread across the media.

Estimation of Bacterial Load from Ulcerative Tissue

To determine the bacterial population associated with the ulcerative lesion of fish, the **serial dilution and spread plate method** was performed. Dilutions ranging from 10^{-1} to 10^{-7} were prepared, and 0.1 mL from each dilution was inoculated onto nutrient agar plates. After incubation for 24–48 hours, colonies were counted. The plates that contained between **30–300 colonies** were considered suitable for calculating the colony-forming units (CFU). Plates with heavy growth were recorded as TNTC (Too Numerous to Count), and those with <30 colonies were considered less reliable.

TABLE 1: The observations and calculated bacterial load are summarized in the table below:

Dilution (10^{-x})	Colony Count	Volume Plated (mL)	CFU/mL	Remarks
10^{-1}	TNTC	0.1	—	Too numerous
10^{-2}	TNTC	0.1	—	Too numerous
10^{-3}	225	0.1	2.25×10^{6}	Within countable range
10^{-4}	140	0.1	1.4×10^{7}	Reliable
10^{-5}	65	0.1	6.5×10^{7}	Most appropriate
10^{-6}	35	0.1	3.5×10^{8}	Lower end of range
10^{-7}	18	0.1	1.8×10^{9}	Below 30 (less accurate)

Based on the above, the **10^{-5} dilution plate (65 colonies)** was selected for final calculation, representing an estimated bacterial density of **6.5×10^{7} CFU/mL** from the infected skin tissue.

This high bacterial load indicates a severe infection and supports the observation of ulcerative symptoms in fish.

Pure Culture Isolation

From the 10^{-5} dilution plate (65 colonies), well-isolated colonies were picked and streaked onto fresh nutrient agar plates. After incubation at 28–30 °C for 24–48 hrs, pure single colonies were obtained. These were sub-cultured onto nutrient agar slants and conducted gram staining process.

Gram Staining Results

Microscopic observation of Gram-stained smears showed a pleomorphic cell population of Gram-positive bacteria (purple-coloured cells).

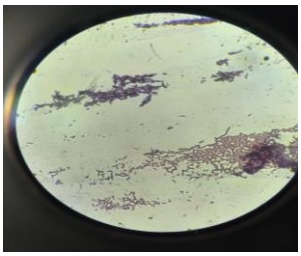
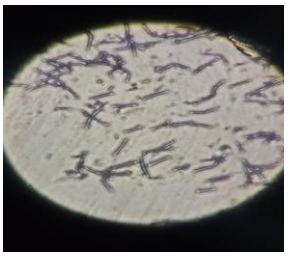
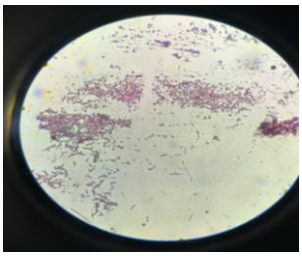

Fig14,15&16: Gram-positive rod shaped(bacilli) & cocci

Bacteria-1

- Gram reaction: Gram positive
- Bacteria morphology: Bacilli (rod shaped)
- In infected fish, gram positive bacilli can be associated with bacteria skin lesions and fin infection diseases.

Bacillus spp. – Large rod-shaped cells that appear singly or in brief chains; opportunistic fish pathogens, often isolated in association with environmental contamination.

Bacteria-2

- The Gram stain also revealed spherical Gram-positive cocci (purple-coloured round cells).
- These cocci appeared in clusters and occasionally in chains.
- Clustered cocci are generally linked with Staphylococcus spp., while chain formation resembles Streptococcus spp.
- Cocci are significant since some species are important fish pathogens (e.g., streptococcus's), whereas others may be part of normal flora.

Streptococcus spp. – Small, round cocci that appear in chains; primary fish pathogens that have been shown to cause ulcerative lesions, haemorrhage, and septicaemia.
The presence of both Bacillus and Streptococcus is evidence of a polymicrobial Gram-positive infection, as can be seen with the clinical presentation on *Channa striata*. Both bacteria have been known to create ulcerative and haemorrhagic conditions, particularly under hygienic compromise and stress conditions.

Discussion

In the field survey at the Siddipet Integrated Fish Market, most of the *Channa striata* (Murrel) were diseased. They had visible indications of bacterial infection. Lesions on the external side included spots with bleeding, open sores, fin rotting, scale loss, and localized skin deterioration. There were some fish that walked slowly and swam abnormally, representing malaise. These symptoms are characteristic of bacterial septicaemia in freshwater fish that is reared and marketed under poor conditions (Muhammad et al., 2020; Patel et al., 2020). Crowding of live fish in unclean tubs of stale

water, combined with continuous contact with waste, blood, and slime during market exposure, fostered microbial proliferation. In addition, incorrect handling during harvesting, transportation, and selling, e.g., failure to wear protective gloves, rough handling, and temperature fluctuation, raised stress levels and made the fish susceptible to opportunistic pathogens (Ibrahim et al., 2014).

Laboratory diagnosis agreed with field findings. Skin and gill samples collected from infected fish cultured on Nutrient Agar developed circular, creamy-white colonies in 24 to 48 hours. Gram staining showed that Gram-positive bacteria rod-shaped Bacillus spp. and cocci in chains (Streptococcus spp.). Mixed bacterial growth was observed in most of the samples, which verified polymicrobial infections. The same findings have been documented in previous research where Bacillus spp., and Streptococcus spp. were common in diseased freshwater fish sold at open markets (Sichone et al., 2013; Dutta et al., 2014). These combined infections result in serious tissue damage, reduced market quality, and increased post-harvest mortality (Mhlanga et al., 2010).

Streptococcus spp. is regarded as a serious bacterial challenge to freshwater aquaculture. They induce ulcerative lesions, septicaemia, and excessive mortality in fish such as tilapia, catfish, and Murrel (Mhlanga et al., 2010). Bacillus spp., commonly utilized as probiotics in controlled hatcheries, have the potential to be pathogenic under stress and unhygienic conditions and can cause fin erosion, bleeding, and skin rot. Their isolation in this study indicates that they can potentially cooperate to exacerbate the severity of the disease, which can accelerate disease progression and make treatment challenging (Patel et al., 2020). The same results have been found in market fish from Zimbabwe and Nigeria, where the predominant bacteria responsible for skin infections were Streptococcus and Bacillus (Ibrahim et al., 2014).

Public health-wise, isolation of Streptococcus spp. and Bacillus spp. from market fish is alarming. Streptococcus spp. is able to infect humans and lead to skin infections, cellulitis, and systemic disease through handling or ingestion of raw or undercooked fish (Matthews et al., 2017). Just like toxic strains of Bacillus spp. have been linked with food poisoning and gastrointestinal diseases in humans (Dutta et al., 2014). The occurrence of these pathogens in market fish shows not just the losses in aquaculture but also potentially severe food safety hazards for consumers. Past studies from India, Southeast Asia, and Africa have suggested that hygienically poor fish markets are points of accumulation for foodborne pathogens such as E. coli, Salmonella, and Streptococcus, which can easily be transmitted to man (Sichone et al., 2013; Muhammad et al., 2020).

The conclusion of this research confirms previous findings that illustrate poor post-harvesting practices as a primary source of bacterial contamination in fresh fish. Poor sanitation, dirty water utilized for live fish, and hand handling without hygiene requirements introduce faecal coliforms and opportunistic bacteria into fish tissues (Dutta et al., 2014). The contamination reduces fish quality, increases spoilage risk, and minimizes shelf-life, impacting consumer health and market economics.

At large, this study reveals that overcrowding, improper hygiene, and unsatisfactory handling procedures were the primary factors behind infection in Murrel fish at Siddipet market. The major bacteria found were Bacillus spp. and Streptococcus spp., usually as combined infections that augmented disease severity. The study emphasizes the immediate need for adopting Good Hygienic Practices and Good Market Practices. Training fish vendors in safe handling, constant checks on microbial levels in fish markets, and water quality management are crucial to minimize contamination risks. These measures will enhance the health of fish and safeguard consumers against possible infections (Matt).

Conclusion

The current research on infected *Channa striata* (Murrel) of Siddipet Integrated Fish Market established that bacterial disease is an issue of concern on fish health, market price, and consumer safety. Observation in the field indicated general symptoms of disease including ulcers, haemorrhage, fin rot, and tissue injury that were directly associated with poor hygienic practices, overcrowding, and stressful handling practices in the market. Culture and Gram stain laboratory analyses revealed the most common pathogens to be Gram-positive Bacillus spp. and Streptococcus spp. and occurring frequently in combination as mixed infections.

The susceptibility of such fish towards the opportunistic bacteria indicates that the infection is not only caused by the primary pathogens but also supplemented by opportunistic pathogens during stress conditions. Such polymicrobial infection facilitates ease of disease control and enhances risk of outbreak of diseases at a quicker rate. Additionally, zoonotic potential of bacteria such as Streptococcus poses a public health hazard, particularly when handled in an unhygienic manner or ingested in an uncooked state.

In summary, the study emphasizes the necessity of enhanced sanitary practices, correct handling procedures, routine microbial analyses, and customer education in fish markets. Through interventions in such matters, the health of the fish as well as food safety can be assured to promote sustainable aquaculture and protection of public health.

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