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Identification of *Salmonella* sp. in Milkfish (*Chanos chanos*) Collected from PPI Ujong Baroh, West Aceh

Identifikasi Bakteri Salmonella sp. pada Ikan Bandeng (Chanos chanos) yang Didaratkan dari PPI Ujong Baroh, Aceh Barat

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Abstract

Milkfish (*Chanos chanos*) is an important fishery commodity in Indonesia due to its high nutritional value and affordability. However, its high moisture and protein content make it highly susceptible to microbial contamination, including pathogenic bacteria such as *Salmonella* sp., which may pose serious food safety risks to consumers. This study aimed to detect and characterize *Salmonella* sp. in milkfish landed at the Ujong Baroh Fish Landing Base (PPI Ujong Baroh), West Aceh Regency, Indonesia. The study was conducted in April 2025 using an observational sampling approach followed by laboratory-based microbiological analysis. A total of 10 milkfish samples were collected purposively during fish landings. Samples were aseptically dissected, and tissues from the gills, skin, muscle, and intestine were homogenized for bacterial analysis. Isolation was carried out using Buffer Peptone Water (BPW) as a pre-enrichment medium and Salmonella Shigella Agar (SSA) as a selective medium. Identification was performed based on macroscopic colony morphology and microscopic Gram staining characteristics. The results showed that all ten samples tested positive for *Salmonella* sp. contamination. Colonies grown on SSA medium exhibited circular shapes, black pigmentation, smooth surfaces, entire margins, and convex elevations. Microscopic observations revealed Gram-negative coccobacillus to be short rod-shaped bacteria that appeared pink after Gram staining. These findings indicate that milkfish landed at PPI Ujong Baroh were contaminated by *Salmonella* sp., highlighting the need for improved sanitation practices and stricter microbiological monitoring during post-harvest fish handling.

Keywords: *Chanos chanos*, Food safety, PPI Ujong Baroh

Abstrak

Ikan bandeng (*Chanos chanos*) merupakan salah satu komoditas perikanan penting di Indonesia karena memiliki nilai gizi tinggi dan harga yang relatif terjangkau. Namun, kandungan protein dan kadar air yang tinggi menyebabkan ikan bandeng mudah mengalami kontaminasi mikroba, termasuk bakteri patogen seperti *Salmonella* sp. yang berpotensi menimbulkan risiko keamanan pangan bagi konsumen. Penelitian ini bertujuan untuk mendeteksi dan mengkarakterisasi keberadaan *Salmonella* sp. pada ikan bandeng yang didaratkan di Pangkalan Pendaratan Ikan (PPI) Ujong Baroh, Kabupaten Aceh Barat. Penelitian dilakukan pada April 2025 menggunakan metode observasional yang dilanjutkan dengan analisis mikrobiologi di laboratorium. Sebanyak 10 sampel ikan bandeng diambil

secara purposive saat aktivitas pendaratan ikan berlangsung. Sampel dibedah secara aseptik dan jaringan insang, kulit, daging, serta usus dihomogenkan untuk analisis bakteriologis. Isolasi bakteri dilakukan menggunakan media pra-pengayaan Buffer Peptone Water (BPW) dan media selektif Salmonella Shigella Agar (SSA). Identifikasi dilakukan berdasarkan karakteristik makroskopis koloni dan pengamatan mikroskopis melalui pewarnaan Gram. Hasil penelitian menunjukkan bahwa seluruh sampel (100%) positif terkontaminasi *Salmonella* sp. Koloni bakteri pada media SSA menunjukkan bentuk bulat, berwarna hitam, permukaan halus, tepi rata, dan elevasi cembung. Pengamatan mikroskopis menunjukkan bakteri berbentuk kokobasil hingga basil pendek yang berwarna merah muda setelah pewarnaan Gram, yang mengindikasikan bakteri Gram negatif. Temuan ini menunjukkan bahwa ikan bandeng yang didaratkan di PPI Ujong Baroh telah terkontaminasi *Salmonella* sp., sehingga diperlukan peningkatan sanitasi dan pengawasan mikrobiologis pada tahap penanganan pascapanen ikan.

Kata kunci: *Chanos chanos*, Keamanan pangan, PPI Ujong Baroh

1. Introduction

West Aceh Regency is one of the coastal regions in Aceh Province, Indonesia, with a coastline extending approximately 50.55 km and a marine area covering about 80.88 km². This region is characterized by diverse coastal ecosystems and substantial marine fishery resources, contributing significantly to local fishery production (Rizal et al., 2011). The fisheries sector, therefore, plays a vital role in sustaining coastal livelihoods and regional economic development. To support fisheries activities, West Aceh operates a Fish Landing Base, namely Ujong Baroh Fish Landing Base (PPI Ujong Baroh), located in Johan Pahlawan District and managed under the Technical Implementation Unit (UPTD) of the Department of Marine Affairs and Fisheries of West Aceh Regency. This facility serves as a central point for landing and distributing various fish species, including tuna, mackerel, snapper, grouper, and milkfish. As the initial nodes in the post-harvest distribution chain, fish landing sites serve as critical control points for maintaining seafood quality and safety.

Milkfish (*Chanos chanos*) is one of Indonesia's leading aquaculture and fisheries commodities due to its high nutritional value and affordability. It serves as an important source of animal protein, containing vitamin A (150 IU), vitamin B1 (0.05 mg), and vitamin C (0 mg), with approximately 80% of its body being edible (Abriana et al., 2021). However, its high protein and moisture content make milkfish highly perishable and particularly susceptible to microbial contamination. As a perishable food product, milkfish is vulnerable to spoilage microorganisms and pathogenic bacteria, which can compromise product quality, reduce shelf life, and pose risks to consumer health (Putra, 2022).

Pathogenic bacteria are microorganisms capable of causing infection and disease in their hosts, potentially inducing tissue alterations through genetic and cellular mechanisms (Suharni et al., 2008). Among the pathogenic bacteria frequently associated with fishery products are *Vibrio* and *Salmonella* sp. Ihsan et al. (2018) reported the presence of *Salmonella* sp. in milkfish sold in traditional markets. Although *Salmonella* sp. is not pathogenic to fish, it is a major foodborne pathogen in humans and may cause salmonellosis when contaminated seafood is consumed (Darmawan et al., 2014). From a regulatory perspective, food safety standards at both national and international levels, including Indonesian National Standards (SNI) and Codex Alimentarius guidelines, generally require the absence of *Salmonella* in ready-to-consume food products. The detection of *Salmonella* in fresh fish, therefore, represents a serious public health concern and indicates potential weaknesses in hygiene practices, handling procedures, sanitation systems, or cold chain management during post-harvest processing. The implementation of Hazard Analysis and Critical Control Points (HACCP) principles emphasizes the importance of monitoring microbial contamination at critical points, particularly at landing sites where fish are first unloaded and distributed.

Despite previous reports of *Salmonella* contamination in milkfish marketed in traditional markets, scientific data regarding the occurrence and microbiological characteristics of *Salmonella* sp. in milkfish directly at fish landing facilities remain limited, particularly at PPI Ujong Baroh in West Aceh. Most existing studies focus on retail markets rather than the upstream stage of the distribution chain. This gap underscores the need for systematic microbiological assessment at the landing stage to establish baseline data for risk mitigation, strengthen HACCP-based monitoring systems, and support evidence-based policy for seafood safety management. Therefore, investigating the presence and characteristics of *Salmonella* sp. in milkfish landed at PPI Ujong Baroh is essential to enhance food safety assurance, protect public health, and contribute to sustainable fisheries management in West Aceh.

2. Material and Method

2.1. Time and Place

This study was conducted in April 2025. Milkfish samples were collected from the Ujong Baroh Fish Landing Base (PPI Ujong Baroh), West Aceh Regency, Aceh Province, Indonesia. Laboratory analyses were subsequently carried out following sample collection.

2.2. Methods

This study employed an observational approach using purposive sampling techniques based on the availability of milkfish landed at PPI Ujong Baroh. A total of 10 fresh milkfish samples were collected during active fish trading hours between 07:00 and 10:00 AM. Before sampling, all required materials were prepared, including sterile sample containers, sterile plastic bags, gloves, 70% alcohol, and a cool box containing ice. Samples were collected aseptically and immediately stored in the cool box to maintain freshness during transportation to the laboratory. The sampling procedure referred to the method described by Ihsan et al. (2018).

2.3. Procedures

2.3.1. Sample Preparation and Sterilization of Equipment

In the laboratory, each fish sample was aseptically dissected. Tissue samples were collected from the gills, skin, muscle, and intestine. These tissues were then combined and homogenized using a sterile mortar to obtain representative samples for microbiological analysis. Laboratory equipment, such as forceps, scissors, dilution bottles, and Erlenmeyer flasks, was wrapped in aluminum foil and sterilized in an autoclave at 121°C under 15 psi for 30 minutes. Petri dishes were cleaned with alcohol, wrapped in sterile paper, sealed in plastic bags, and sterilized under the same conditions.

2.3.3. Preparation of Culture Media

A total of 4 g of Buffer Peptone Water (BPW) was weighed using an analytical balance and dissolved in 200 mL of distilled water in an Erlenmeyer flask. The solution was homogenized and sterilized in an autoclave at 121°C for 15 minutes. A total of 12.6 g of Salmonella Shigella Agar (SSA) powder was dissolved in 200 mL of distilled water. The mixture was heated on a hot plate stirrer at approximately 125°C, with continuous stirring, until completely homogenized. The prepared medium was then aseptically poured into sterile Petri dishes under laminar airflow conditions.

2.3.4. Bacterial Enrichment and Isolation of Salmonella sp.

Approximately 2.2 g of homogenized fish tissue was transferred into a sterile stomacher bag containing 200 mL of BPW. The mixture was homogenized and incubated at 35°C for 24 hours to enrich for bacteria. Following enrichment, one loopful of bacterial suspension was streaked onto SSA media and incubated at 35°C for 24 hours. Presumptive *Salmonella* colonies were identified based on their characteristic morphology on SSA medium, as described by Ihsan et al. (2018).

2.3.5 Gram Staining and Microscopic Identification

Gram staining was performed following the method described by Amiruddin et al. (2017). Suspected colonies were smeared onto sterile glass slides and subjected to crystal violet staining, Lugol's iodine treatment, alcohol decolorization, and safranin counterstaining. The stained samples were observed under a microscope at 100× magnification to determine bacterial morphology and Gram characteristics.

2.4. Data Analysis

All data obtained from laboratory observations were tabulated and presented in tables and figures. The results were analyzed using a descriptive approach to provide a systematic interpretation of the findings.

3. Result and Discussion

3.1. Detection of Salmonella sp.

Salmonella sp. is a pathogenic bacterium recognized as one of the primary causes of foodborne diseases (Kuswiyanto, 2017). The results of the detection test for Salmonella sp. in milkfish samples collected from the Ujong Baroh Fish Landing Base (PPI Ujong Baroh), West Aceh Regency, are presented in Table 1. The analysis revealed that all examined samples tested positive for *Salmonella* sp., indicating contamination in each.

Based on observations using the selective medium Salmonella Shigella Agar (SSA), all 10 milkfish samples collected from PPI Ujong Baroh tested positive for *Salmonella* sp. Identification was indicated by the growth of black colonies on SSA medium. According to Ihsan et al. (2018), *Salmonella* sp. colonies grown on SSA typically appear pink with a black center, circular in shape, convex in elevation, smooth in texture, with entire margins, and opaque. Similarly, Femila et al. (2018) reported that colonies exhibiting pink coloration, a circular shape, a

medium size, a soft consistency, entire margins, a smooth surface, and convex elevation are characteristic of *Salmonella* sp.

Table 1. Results of *Salmonella* sp. detection in milkfish samples

Sample	Result	Description
1D	+	Contaminated
2D	+	Contaminated
3D	+	Contaminated
4D	+	Contaminated
5D	+	Contaminated
6P	+	Contaminated
7P	+	Contaminated
8P	+	Contaminated
9P	+	Contaminated
10P	+	Contaminated

A bacterial colony represents a visible cluster of bacterial cells that arises from the multiplication of a single bacterium on a solid medium. The morphological characteristics of colonies, including color, shape, size, texture, and elevation, vary depending on the type of medium used and the bacterial species present.

3.2. Macroscopic Characteristics

Macroscopic observations were conducted to examine the growth characteristics of *Salmonella* sp. following inoculation from Buffer Peptone Water (BPW) enrichment medium onto Salmonella Shigella Agar (SSA). The resulting colony morphology on SSA was observed and documented (Figure 1).

Figure 1. Bacterial colonies on Salmonella Shigella Agar (SSA) medium

3.3. Macroscopic Colony Characteristics on SSA Medium

The growth of *Salmonella* sp. on Salmonella Shigella Agar (SSA) demonstrated colonies that were circular in shape, smooth in texture, glossy in appearance, with entire margins and a distinct black center (Jadhey et al., 2020). The black pigmentation observed in the colonies is associated with the bacterium's ability to produce hydrogen sulfide (H_2S) gas. SSA medium permits the growth of colonies exhibiting black, pink, or yellow coloration. Yellow colonies generally indicate bacteria belonging to the genus *Shigella*, whereas black- or pink-colored colonies are characteristic of the genus *Salmonella*. The selectivity of the SSA medium is attributed to its main components, including lactose, peptone, bile salts, ferric citrate, and the indicator neutral red.

Bacteria of the genus *Shigella* are unable to ferment lactose, do not produce H_2S gas, and lack the enzyme thiosulfate reductase; therefore, the resulting colonies appear yellow or colorless (Aini, 2018). In contrast, *Salmonella* does not ferment lactose but produces hydrogen sulfide (H_2S), resulting in colorless colonies with a black center. The black precipitate observed on the SSA medium indicates H_2S production by *Salmonella* sp., a distinguishing characteristic from *Shigella* (Aryani et al., 2025).

Based on macroscopic observations in this study, colonies grown on SSA exhibited morphological characteristics consistent with *Salmonella* sp. The colonies were circular, convex, smooth-surfaced, and displayed a black center. In addition, pink colonies with circular shape, medium size, soft consistency, entire margins, smooth surface, and convex elevation were also observed, consistent with the characteristics of *Salmonella* sp. as described by Femila et al. (2018). Overall, the macroscopic characteristics observed on the SSA medium strongly support the presumptive identification of *Salmonella* sp. in the milkfish samples.

3.4. Microscopic Characteristics

Gram staining was performed to observe the microscopic characteristics of *Salmonella* sp. The examination was conducted using a microscope under 100× magnification. This method allows observation of bacterial morphology, including cell shape, size, and staining characteristics, following the Gram staining procedure. The

microscopic observations, including the morphological features and staining results of the bacterial cells, are presented in Figure 2.

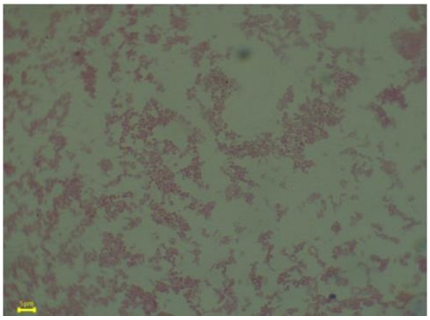

Figure 2. Bacterial cells observed under 100× magnification following Gram staining

Microscopic examination revealed bacterial cells with coccobacillary morphology that stained pink with Gram stain. The pink coloration is associated with the high lipopolysaccharide content in the outer membrane of Gram-negative bacteria. During the decolorization step using 90% alcohol, the crystal violet–iodine complex is removed due to the disruption of the lipid-rich outer membrane. As a result, the primary stain (crystal violet) is washed out, and the cells subsequently take up the counterstain (safranin), producing a pink or red appearance under the microscope.

The pink coloration observed under a microscope indicates that the bacteria belong to the Gram-negative group (Amiruddin et al., 2017). Based on microscopic examination of bacterial colonies from all 10 samples, consistent morphological characteristics were observed during Gram staining. The bacteria exhibited rod-shaped (bacilli) or short bacilli (coccobacilli) morphology, red or pink coloration, scattered arrangement, and Gram-negative properties. On Salmonella Shigella Agar (SSA), pink colonies corresponded microscopically to rod-shaped bacteria with red coloration, scattered arrangement, and Gram-negative characteristics (–). Similarly, black colonies showed short rod-shaped bacteria, red coloration, scattered arrangement, and Gram-negative properties (–), consistent with the characteristics of *Salmonella* sp. (Femila et al., 2018).

4. Conclusions

Based on the detection test of *Salmonella* sp. in milkfish collected from the Ujong Baroh Fish Landing Base (PPI Ujong Baroh), West Aceh Regency, a total of 10 samples were examined. All samples tested positive for the presence of bacteria belonging to the genus *Salmonella* sp. This was confirmed through both macroscopic and microscopic characteristics consistent with *Salmonella* sp. *Salmonella* sp. is a Gram-negative pathogenic bacterium. In the liquid enrichment medium, Buffer Peptone Water (BPW), bacterial growth was indicated by a change from clear yellow to turbid yellow. On the selective agar medium, Salmonella Shigella Agar (SSA), incubation for 24 hours resulted in the appearance of circular colonies with black centers and pink coloration.

Furthermore, microscopic observation at 100× magnification revealed coccobacillus-shaped, short-rod bacterial cells with variable lengths (short, medium, and elongated forms). These morphological characteristics are consistent with the identification of *Salmonella* sp. Overall, the findings confirm contamination of milkfish landed at PPI Ujong Baroh by *Salmonella* sp., indicating potential food safety risks and emphasizing the importance of improved sanitation and monitoring practices at the fish landing stage.

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