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Original article

Assessment of *Escherichia coli* Contamination in Catfish (*Clarias gariepinus*) Sold at Traditional Markets in Blitar, Indonesia

Fariska Amadea Julia[1], Arif Mutaqqin[2], Lisa Lisdiana[1*]

[1] Undergraduate Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya 60231, Indonesia
[2] Fish Health and Aquaculture Environment Laboratory, Department of Animal Husbandry and Fisheries of Blitar Regency, Blitar City 66117, Indonesia

Abstract

Catfish is easy to cultivate and is in high demand by consumers. Traditional markets are one of the main places in Blitar for buying and selling catfish. However, catfish are susceptible to infection caused by *E. coli*. Therefore, the level of *E. coli* as an indicator of catfish hygiene in two traditional markets in Blitar needs to be studied. This research employed a descriptive method that included interviews, sampling, and testing the level of *E. coli* using the MPN. The result indicated the average level of *E. coli* contamination on catfish at Pon Market was 13.8 MPN/g, while at Templek Market was 9.13 MPN/g. Both of them exceed the threshold set by SNI 7388-2009, which stated the maximum limit for fresh fish's *E. coli* level is <3 MPN/g. The average level of *E. coli* contamination at Pon Market is higher than Templek Market due to differences in the catfish handling by sellers after being taken from catfish cultivator. Prolonged storage of catfish in insufficient water conditions can deteriorate their health, making them more susceptible to infections such as *E. coli*. Poor equipment and environmental hygiene conditions are also contributes to the risk of catfish infection by pathogenic bacteria that cause catfish to be potentially infected by pathogenic bacteria. Therefore, sellers must pay more attention to catfish hygiene and handling so that it is free from *E. coli* contamination. Also, the consumers should cook the catfish properly to prevent the transmission of *E. coli*.

Keywords: catfish, *E. coli*, hygiene, MPN, traditional market

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Introduction

Indonesia is a maritime nation with vast coastal and marine areas, which contribute to its diverse range of natural resources, including marine and freshwater fish. One popular freshwater fish in Indonesia is catfish, which is widely cultivated in this country (Rozi *et al*., 2020). Catfish are easy to cultivate, have a delicious taste, and are high in nutritional content. Catfish contain nutrients such as omega-3 and omega-6 fatty acids, as well as lysine and leucine (Kartikasari *et al*., 2018). Lysine and leucine are essential for growth and development (Andri *et al*., 2020). In addition to its nutritional value, catfish is also relatively affordable and readily available in markets (Anisa *et al*., 2022). Consequently, catfish are in high demand across Indonesia, including in the Blitar region

According to the BPS-Statistics Indonesia in Blitar, catfish production continued to increase from 2020 to 2023. In 2023, the public consumption rate reached 111,490 fish. Consumers can purchase catfish directly from catfish cultivator or at the market. Markets are categorized into two types, traditional markets and modern markets. Modern markets provide services and goods with high quality and service to consumers and are managed with modern management (Sari *et al*., 2025). Fish handling in modern markets adheres to product quality standards, ensuring hygiene. Traditional markets, on the other hand, are managed by the government, local governments, and in partnership with the private sector, such as kiosks, shops, or tents operated by small communities (Sahban & Perwira, 2018). Traditional markets are one of the main places for residents of Blitar to transact goods, including catfish.

Pon Market and Templek Market in Blitar are traditional markets that sell a variety of fish, from freshwater to marine fish. One of the fish commonly sold at Pon Market and Templek Market is catfish. The catfish handling methods at both markets differ. One vendor at Pon Market sells approximately 50 kg of catfish each day. If the catfish are not sold on that day, they are stored in a small pond located under the vendor's table and sold the following day. The water source for this pond comes from the market's water supply. Meanwhile, a vendor at Templek Market sells 90 kg of catfish daily, and the stock is always sold out at the end of the day. The vendor does not add any water to the catfish tank, instead, the water comes from the fish cultivator. The similarity in catfish handling between the vendors is that they source the catfish from the cultivator the day before selling them at the market.

Catfish consumed by consumers requires careful attention to the quality and hygiene levels. Food that is not properly hygienic can cause various health problems, such as diarrhea, dysentery, cholera, poisoning, and even disrupt the growth and development of children (Fitri *et al.*, 2022). Contamination in catfish for consumption can be influenced by factors such as the cultivation environment, the quality of animal feed, the processing and distribution methods of the fish, and fish handling by vendors. Contamination in fish can be microbes, such as

* Corresponding Author:
Lisa Lisdiana
Undergraduate Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya 60231, Indonesia
Phone: +6281326370488
E-mail: lisalisdiana@unesa.ac.id

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fungi, bacteria, or viruses. One of the bacteria that frequently infects catfish is *Escherichia coli*. According to SNI 7388-2009, the maximum limit for *E. coli* bacteria in fresh fish is <3 MPN/g

Previous studies have also found *E. coli* contamination in fish. In a study conducted by Larawo *et al*. (2024), *E. coli* contamination was found in Grouper fish, water used for handling Grouper fish, and ice used to preserve Grouper fish, which exceeded the threshold. Moreover, in the study conducted by Fauzi *et al.* (2024) found *E. coli* contamination in fresh shrimp and dory fish samples, namely more than 3 MPN/g. In a study conducted by Fitri *et al.* (2022), *E. coli* contamination was found in smoked catfish at the Traditional Market in Simpang Kiri District, Subulussalam City.

To date, there has been no published research on *E. coli* contamination in catfish specifically from Pon Market and Templek Market in Blitar. *E. coli* contamination that is not properly handled by the government, seller, or fish cultivator will certainly be very detrimental to other parties. Based on the above description, the level of *E. coli*, which is an indicator of catfish hygiene in two traditional markets in Blitar, needs to be studied.

Methods

Type of Research

This research is a descriptive study with qualitative and quantitative approaches. Sampling was conducted at Templek Market and Pon Market in April 2025. Data collection methods included interviews, sampling, and testing the level of *E. coli* using the Most Probable Number (MPN) method.

Sampling

The selected markets are centrally located in Blitar, thus, numerous residents can easily access the market and buy the catfish. Samples were taken from two traditional markets in Blitar using a random sampling technique, which involves randomly selecting fish sellers from a population without regard to strata (Fajar *et al*., 2021). Selected traditional markets were Pon Market and Templek Market (Figure 1). Samples were transported in a cool box to prevent contamination during transportation. Upon arrival at the laboratory, the catfish were immediately tested for *E. coli*. Interview to the selected sellers was also performed. Each of the sellers were given eight open questions related to fish resource and handling (Table 1).

Fig. 1. Location of Pon Market and Templek Market areas

Tools and Materials

The materials used in this study include: catfish samples, PBS (Phosphate Buffered Saline) media (Himedia *cat#20971-500G*), Lauryl Triphtose Broth (LTB) media (Himedia *cat#M080-500G*), *E. coli* Broth (ECB) media (Himedia *cat#M1271-500G*), Eosin Methylene Blue Agar (EMBA) media (Himedia *cat#M317-500G*), distilled water, and 96% alcohol. Meanwhile, the tools used include: cool box, scissors, knife, tray, digital scale, aluminum foil, mortar and pestle, hot plate, erlenmeyer flask, sterile spoon, stirrer, test tube, test tube rack, beaker glass, measuring cup, cotton, autoclave, refrigerator, incubator, petri dish, LAF, tip, micropipette, vortex, Durham tube, sterile inoculating loop, and label.

Sample Preparation

Upon arrival at the laboratory, the catfish skin was sterilized using alcohol 70% to eliminate the surface microorganisms. This study using three catfish samples for repetition. 2.5 grams of meat and skin from each of three catfish samples were collected. The meat and skin of three catfish samples were ground using a mortar and pestle, separately After the meat and skin of catfish were homogenized, it was added and homogenized using a vortex to get a 10^{-1} dilution. Then the dilution of 10^{-2} was prepared by adding 1 ml of dilution 10^{-1} to 9 ml of PBS and homogenizing them using a vortex. The dilution series was continued until getting a 10^{-3} dilution with the same process. The MPN method was used to determine the level of *E. coli* in catfish. MPN method consists of the coliform presumptive test, the *E. coli* prediction test, and the *E. coli* confirmation test.

Coliform Presumptive Test

The coliform presumptive test was performed by transferring one ml of each sample dilution into three series of LTB media tubes containing Durham tubes using a micropipette. Then, the tubes were incubated for 48 hours in an incubator at 35°C. A positive tube is indicated by the presence of turbidity and gas formation in the Durham tube. If the tube is positive, it was then proceeded to the *E. coli* presumptive test.

E. coli Presumptive Test

The positive tubes from the coliform presumptive test were transferred to tubes containing ECB using a sterile ose needle. Then, the ECB tubes were incubated for 48 hours at 45°C. Test tubes with increasing turbidity and formation of gas in the Durham tubes are indicated as positive, and then proceeded for the *E. coli* confirmation test.

E. coli Confirmation Test

The *E. coli* confirmation test was performed by plating culture from the positive tube of the *E. coli* presumptive test into EMBA medium with a sterile inoculating loop. The inoculated media was then incubated in an incubator at 35°C for 24 hours. *E. coli* colonies are characterized by a black center or metallic green color (Cartas *et al*., 2022). Positive inoculation for each dilution results were recorded and compared with the MPN index table to determine the *E. coli* count.

Data Analysis

The obtained data were analyzed using the MPN method. The MPN method is a statistical method used to estimate the number of variable microorganisms in a sample, based on growth in a series of tubes containing liquid or solid media. The series of three tubes was used and the data results were compared with the MPN index table in SNI 01-2332.1 of 2006.

Results and Discussion

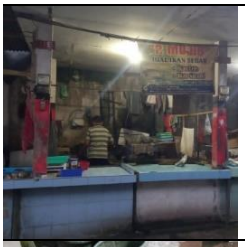
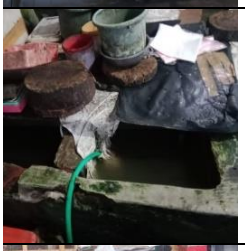
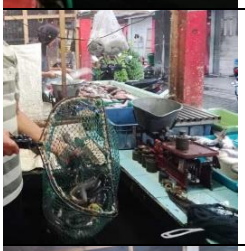
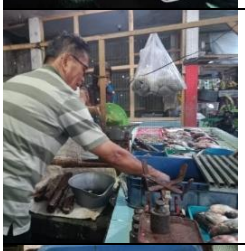
The fish species studied was the dumbo catfish from two markets. Catfish from both markets were sourced from different catfish cultivators. Catfish from Pon Market were obtained from a catfish cultivator in Gandusari District, while catfish from Templek Market were obtained from Kademangan District. Dumbo catfish are often cultivated by catfish cultivator due to their high adaptability, short rearing period (approximately 3 months), disease resistance, and ease of cultivation (Rizky *et al*., 2024). According to Rizky *et al*. (2024), demand for dumbo catfish is high because of its high nutritional value. Based on interviews, the seller at Pon Market consumes approximately 50 kg of catfish daily, while the seller at Templek Market consumes approximately 90 kg of catfish daily (Table 1).

Table 1. Results of interviews with catfish sellers in Pon Market and Templek Market

Category	Pon Market	Templek Market
Product variations sold by Fish seller	The seller sells various types of fish	The seller only sells catfish.
Number of catfish sold per day	Approximately 50 kg	Approximately 90 kg
Time of catfish collection	One day before sale	One day before sale
Type of catfish	Dumbo catfish	Dumbo catfish
Origin of cultivator catfish	Gandusari	Kademangan
Types of ponds for catfish cultivation (at fish cultivator / supplier)	Conventional concrete ponds	Conventional concrete ponds
Catfish handling	Unsold catfish were placed in a small pond under the trading table and sold the next day	The catfish are always sold out within one day
Water sources	Regional water company (PDAM)	Water from the cultivator pond

The catfish from the two catfish cultivators shared a similarity, they are cultivated in conventional concrete ponds (Table 1). Concrete ponds are a permanent structure constructed primarily from sand and cement. Many cultivators choose concrete ponds because they are more durable and leak-proof than earthen or tarpaulin ponds. Furthermore, concrete ponds are considered more hygienic and water-efficient (Harizahayu *et al*., 2021). The quality of the water source directly affects the quality of the catfish. Therefore, the water source used for cultivation should be carefully considered to guarantee the quality of the catfish.

Table 2. Condition of one of the Catfish Sellers at Pon Market and Templek Market

Pon Market	Templek Market	Information
		Condition of the catfish seller's selling place
		Place to put catfish before putting it on the table
		Tool for taking catfish from a small pond (Pon market) or drum (Templek market)
		Catfish weighing process
		Condition of catfish on the table (uncovered)

During sample preparation, all equipment used was ensured to be sterilized, including the catfish. The skin of the catfish was sterilized using alcohol 70%. This is to eliminate microorganisms on the surface of the skin. Alcohol is effective in killing microorganisms in living tissue (Marthisza *et al*., 2025).

In the coliform presumptive test, LTB media is used. LTB media is a medium used for the cultivation of coliform. Positive tubes are indicated by the increasing turbidity of LTB media and the gas formation in the Durham tube. Meanwhile, negative results are indicated by constant turbidity of the media and no gas formation in the Durham tube (Figure 2). The gas formation in the Durham tube is produced from the fermentation of lactose by coliform bacteria. During this process, the bacteria use lactose as a carbon source, which leads to the production of gas (Saridewi *et al*., 2016). The number of positive and negative results in each tube series was counted, and then recorded as follows (Table 3).

Fig. 2. Positive result (right) and negative result (left) isolates on LTB

Table 3. Results of Coliform Estimation Tests on LTB

Parameter	Pon Market			Templek Market		
	1	2	3	1	2	3
LTB Planting Results	3-3-0	3-3-0	3-0-1	1-2-0	2-1-0	1-0-2

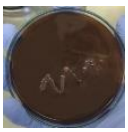
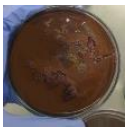
Positive tubes from the coliform presumptive test were further tested for the *E. coli* presumptive test. The medium used for the *E. coli* test was ECB, which a medium used for cultivating *E. coli*. In the *E. coli* presumptive test, inoculated ECB medium was incubated at 45^{O}C for 48 hours. Incubating at 45°C allows for the optimal growth and metabolism of thermotolerant gram-negative bacteria, such as *E. coli* and fecal coliforms, while gram-positive bacteria cannot grow optimally at this temperature (Saridewi *et al*., 2016). Tubes with increasing turbidity and gas production were categorized as positive. The results of the three-tube series were recorded as shown in Table 4**.** Positive tubes were tested for *E. coli* confirmation tested..

Table 4. Results of the *E. coli* Presumptive Test in ECB

Parameter	Pon Market			Templek Market		
	1	2	3	1	2	3
Positif Results	3-0-0	3-0-0	2-0-0	1-2-0	2-1-0	1-0-1

Positive tubes resulted from the *E. coli* presumptive test was further tested by planting on the EMBA medium. *E. coli* colonies are indicated by blackish blue or metallic green colony color, while negative colonies are indicated by white or transparent colors. This is because EMBA media contains lactose, eosin dye, and methylene blue, thereby enabling it to differentiate between lactose-fermenting bacteria and non-fermenters (Cartas *et al*., 2022). Meanwhile, the blackish or metallic green color is caused by the amount of acid produced from the precipitation of the dye on the surface of the bacterial growth. Based on the results listed in Table 5, all six samples of meat and skin of catfish were confirmed to be positive for *E. coli*.

Table 5. Results of the *E. coli* Confirmation Test in EMBA

Sample	EMBA Planting Results	Picture
Pon catfish 1	2-0-0	
Pon catfish 2	3-0-0	
Pon catfish 3	2-0-0	
Templek catfish 1	1-2-0	
Templek catfish 2	2-0-0	
Templek catfish 3	1-0-1	

The number of positive colonies for each sample from EMBA medium was recorded, yielding a three-tube series result. Then, the results were used to determine the level of *E. coli* for each sample by referencing the MPN index table in SNI 01-2332.1 2006. Based on Table 6, the average *E. coli* from Pon Market was higher than that from Templek Market. According to SNI 7388-2009, the maximum permissible level for *E. coli* in fresh fish is < 3MPN/g. Therefore, both the catfish samples from Pon Market and Templek Market failed to meet the specified Indonesian National Standard.

Table 6. Results of the *E. coli* Tests on Catfish from Pon Market and Templek Market

Parameter	Pon Market				Templek Market			
	1	2	3	Average *E. coli* Content (MPN/g)	1	2	3	Average *E. coli* Content (MPN/g)
***E. coli* content (MPN/g)**	9.2	23	9.2	13.8	11	9.2	7.2	9.13
Information	Does not meet standards	Does not meet standards	Does not meet standards		Does not meet standards	Does not meet standards	Does not meet standards	

The presence of *E. coli* in the samples may be associated with by several factors, such as the quality of the water source, the equipment hygiene, catfish handling practices, and environmental sanitation. Observations revealed several similarities and differences in how the two sellers managed the catfish (Table 2). Both sellers collected their catfish from the cultivator the day before selling them. The catfish were transported from the cultivator in plastic containers, such as drums, and held overnight without feeding. The low water volume with a high density of catfish leads to low oxygen levels in the containers. This lack of oxygen condition can increase the risk of disease, induce stress in the fish, and reduce productivity (Andriani & Nurinsani, 2025). These practices, particularly the stressful conditions, put the catfish of Pon Market and Templek Market at an increased risk of *E. coli* contamination. Catfish from Pon Market that are not sold out on the same day are placed in a small pond located under the seller's table. The pond was irrigated with water supplied by the Regional Water Company (PDAM). PDAM water may have the potential to be contaminated with *E. coli*. According to the Drinking Water Supply System Development Support Agency (BPPSPAM) report for year 2013 (Restina *et al*., 2019), found that only 171 of 328 PDAM water samples were considered healthy, while the remainder were classified as unhealthy. According to Wardoyo & Hutapea (2024), reported that both PDAM water and groundwater in Sukoharjo Regency contained *E. coli*. However, report of specific research on the *E. coli* contamination in PDAM (Regional Water Company) water in Blitar still unavailable. Therefore, the findings from BPPSPAM and Marthisza *et al*. (2025) are utilized as circumstantial evidence to support the potential risk that even treated PDAM water still has to be contaminated with *E. coli*.

In contrast, catfish from Templek Market seller is typically sold out the same day, and the water used comes directly from the cultivator. Cultivator use groundwater as their source. However, groundwater also has the potential for *E. coli* contamination, especially if the surrounding environment is contaminated.

The practice of placing catfish in small ponds by the seller at Pon Market led to a deterioration of the fish's conditions with low water and not being fed for some time (Table 2). Signs of catfish quality decline were cuts on all three catfish samples from Pon Market, while only one sample from Templek Market had cuts. Catfish kept in high-density conditions with low water volume expend more energy to survive, which accelerates the process of quality deterioration (Devi, 2022). This decline in quality makes the catfish more susceptible to contamination by pathogenic microorganisms, such as bacteria. Pathogenic bacteria that can cause disease include *E. coli*, *Salmonella typhi, Shigella dysentriae, Aeromonas hydrophila*, *Plesiomonas shigeelloides*, and *Citrobacter freundii* (Angreni *et al.*, 2018).

The tables used by both sellers were made of the same material, concrete covered with ceramic tiles. The equipment used, including the plastic trays for placing the fish on the tables and the aluminium scales, was also similar (Table 2). However, these materials were rarely washed with soap and were only washed with water. Soap is a cleaning agent that can effectively remove dirt and germs (Sianipar & Sijabat, 2021). Cleaning process without the soup may increase the potential of bacterial infection.

The catfish from both markets were placed on uncovered tables (Tables 2). Based on observations, flies were seen alighting on the catfish. Flies are attracted to unhygienic and foul-smelling environments, such as garbage dumps, markets, farms, restaurants, human waste, and carcasses (Tomasowa *et al*., 2024). The environments around both Pon Market and Templek Market contained sources of attraction for flies, including sellers of fish and chicken meat, garbage piles, and waste disposal areas. Flies that are on these unhygienic areas can become vectors, carrying *E. coli* when they alight on catfish on the tables. According to Tomasowa *et al*. (2024), the dominant fly species in traditional markets are *Musca domestica*, *Sarcophaga haemorrhoidalis*, and *Chrysomya megacephala*. These species have the potential to carry *Staphylococcus, E. coli, Salmonella, Shigella, Vibrio, Providencia*, and *Serratia.*

This study had limitations in determining the source of *E. coli* contamination in the catfish samples. The primary issue was the absence of *E. coli* testing from the water environments, both at the farm and during storage before sale. Therefore, for future similar studies, it is recommended that researchers test not only the catfish for *E. coli* but also its environment.

Consuming catfish with *E. coli* levels that exceed the safety limit can cause various health problems in humans. Some diseases caused by *E. coli* include cholera, diarrhea, and various other digestive ailments (Hunowu *et al.*, 2023). The *E. coli* level obtained from each sample exceeded the threshold. The maximum permissible limit for *E. coli* in fresh fish, as set by SNI 7388-2009, is < 3 MPN/g.

Catfish from both markets can still be consumed by consumers, but consumers must cook the catfish thoroughly. This is because *E. coli* can be inactivated at temperatures above 50°C (Saimah *et al.*, 2016). Furthermore, consumers should immediately process catfish purchased from traditional markets and avoid storing the catfish in open areas. This is because storing catfish in open areas where they are exposed to flies will further increase *E. coli* contamination. Furthermore, local governments should increase their outreach to sellers about the importance of implementing sanitation and hygiene during catfish sales to prevent and reduce the risk of *E. coli* contamination.

Conclusion

Based on research conducted, the *E. coli* level of catfish from Pon Market and Templek Market exceeded the maximum permissible set by SNI 7388-2009 for fresh fish, which is < 3 MPN/g. The level of *E. coli* contamination on catfish from Pon Market is higher than Templek Market due to overnight storage caused by unsold catfish stock. Periodic monitoring and community outreach should be implemented to mitigate further *E. coli* infection and transmission from catfish. Therefore,

sellers and local government must pay careful attention to hygiene and proper handling of catfish to ensure that the catfish sold are free *from E. coli* contamination.

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