Bioscientist: Jurnal Ilmiah Biologi
https://e-journal.undikma.ac.id/index.php/bioscientist

June 2026 Vol. 14, No. 2
e-ISSN: 2654-4571
pp. 969-979

Microbiological Quality and Presumptive *Enterobacter* Contamination in Beef Liver Sold at Traditional Markets in Sidoarjo City

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Received: April 2026; Revised: May 2026; Accepted: June 2026; Published: June 2026

Abstract: This study aimed to evaluate the microbiological quality of beef liver sold in traditional markets in Sidoarjo City by determining the total plate count (TPC) of total microorganisms and Enterobacteriaceae, as well as conducting presumptive identification of the genus *Enterobacter* through biochemical testing. The TPC results for total microorganisms showed that 7 of the 10 samples exceeded the maximum limit specified by the Indonesian National Standard (SNI). In addition, all 10 samples exceeded the Enterobacteriaceae limit established in SNI 9159:2023. Biochemical test results indicated that isolate B2, obtained from Porong Market, exhibited characteristics similar to those of *Enterobacter cloacae*, namely indole (–), H_2S (–), motility (+), methyl red (MR) (–), Voges–Proskauer (VP) (+), citrate (+), and urease (+). The high levels of microbial contamination indicate that beef liver samples from traditional markets in Sidoarjo City did not meet the microbiological standards for safe consumption. Therefore, to obtain its nutritional benefits while minimizing the risk of microbial infection, beef liver should not be consumed raw and should be cooked thoroughly at approximately 70°C.
Keywords: Beef liver; total plate count; *Enterobacter*; traditional market; food safety

How to Cite: Agustin, K. D., & Isnawati. (2026). Microbiological Quality and Presumptive Enterobacter Contamination in Beef Liver Sold at Traditional Markets in Sidoarjo City. *Bioscientist: Jurnal Ilmiah Biologi*, *14*(2), 969–979. https://doi.org/10.33394/bioscientist.v14i2.20732

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INTRODUCTION

Beef liver is an animal-derived food product that is widely consumed because of its high nutritional value and broad availability in markets. It is particularly beneficial for individuals with anemia because it contains heme iron, a form of iron that is readily absorbed by the body and is less affected by dietary inhibitory factors (Arima et al., 2019). Compared with chicken liver, beef liver contains higher protein levels and lower fat content (Amertaningtyas et al., 2021). It is also an important source of protein, vitamins A and B12, folic acid, iron, zinc, and purines, all of which contribute to growth and physiological development (Kang et al., 2017).

Despite its nutritional value, beef liver is highly susceptible to microbial spoilage because of its high moisture content, which supports microbial growth. In addition, its anatomical proximity to the gastrointestinal tract during slaughtering and processing may increase the risk of contamination by intestinal microorganisms (Kirrella et al., 2017). *Enterobacter*, a member of the family Enterobacteriaceae, is commonly associated with food spoilage and can serve as an indicator of food safety (Aufani et al., 2023). Wesevich et al. (2020) reported that *E. cloacae* caused bloodstream infections, or bacteremia, in 104 of 150 patients (69%), followed by *E. aerogenes* in 46 of 150 patients (31%). Contamination of beef liver by *Enterobacter* may occur in unhygienic environments, including traditional markets. Such markets are particularly vulnerable to microbial contamination because they are generally open-air facilities and often lack adequate refrigeration systems (Hartanto et al., 2023).

SNI 9159:2023, which regulates microbiological criteria for foods of animal origin, establishes microbiological quality requirements for animal products and their derivatives. According to this standard, beef liver should not exceed an Enterobacteriaceae contamination limit of ≤1 × 10^2 CFU/g and a total plate count (TPC) limit of ≤1 × 10^6 CFU/g. Rahmawati et al. (2021) reported that beef liver samples obtained from traditional markets in Semarang exceeded the microbial safety limit of 10^6 CFU/g stipulated in SNI 7388:2009. Similarly, Bariz & Syaputri (2024) found that 2 of 8 beef samples collected from traditional markets in Bandung were contaminated with *Enterobacter* sp. Nevertheless, studies on *Enterobacter* contamination in beef liver remain limited, particularly those focusing on beef liver sold in traditional markets in Sidoarjo. This research gap is important because *Enterobacter* is an opportunistic pathogen that may pose health risks when contaminated beef liver is handled or consumed under unhygienic conditions.

Beef liver is relatively popular in Sidoarjo because it is affordable and easily accessible. According to 2024 data from the Central Statistics Agency (BPS), the average weekly per capita consumption of beef liver in Sidoarjo City was 13 g (BPS, 2025). Larangan Market and Porong Market are among the largest traditional markets in Sidoarjo City. Both markets regularly sell beef and beef offal, involve a considerable number of vendors, and have an average daily sales volume of more than 25 kg. Therefore, this study aimed to determine the total microbial count and Enterobacteriaceae contamination levels in beef liver samples collected from Larangan Market and Porong Market using the TPC method. This study also aimed to identify presumptive *Enterobacter* contamination in the samples. The TPC results were compared with the microbiological limits specified in SNI 9159:2023 to evaluate the microbiological safety of beef liver sold in traditional markets in Sidoarjo City.

METHOD

This study used an exploratory descriptive design to determine the level of total microbial contamination and Enterobacteriaceae contamination in beef liver samples using the Total Plate Count (TPC) method. Presumptive identification of *Enterobacter* contamination was performed through Gram staining and biochemical tests. Beef liver samples were obtained from traditional markets in Sidoarjo City. The study was conducted from February to March 2026 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University.

Materials and Equipment

The equipment used in this study included a laminar airflow cabinet, microscope, incubator, and autoclave. The materials consisted of beef liver samples, Plate Count Agar (PCA), 0.1% Buffered Peptone Water (BPW), Nutrient Agar (NA), Violet Red Bile Glucose Agar (VRBGA), MacConkey agar (MAC), Sulfide Indole Motility (SIM) agar, Kovac's reagent, MR-VP broth, methyl red, α-naphthol, 40% KOH, Simmons Citrate Agar (SCA), urea agar, distilled water, 70% alcohol, 96% ethanol, crystal violet, Lugol's solution, and safranin.

Sampling and Sample Preparation

Beef liver samples were collected from Larangan Market and Porong Market in Sidoarjo City at 4:00 a.m. A total of five sellers from each market were selected, and 250 g of fresh beef liver was collected from each seller. The samples from the two markets were not obtained from the same batch. Samples from Larangan Market were collected on the first day, whereas samples from Porong Market were collected on the second day. All samples were collected in fresh condition without packaging or freezing

treatment. Microbiological analysis was performed less than 24 hours after sample collection.

Total Microbial and Enterobacteriaceae Counts

A 25 g portion of each beef liver sample was weighed aseptically, placed in a sterile plastic bag, and ground using a sterile mortar and pestle. The 25 g portion consisted of both surface and internal parts of the beef liver. A total of 225 mL of BPW was added to the sample, and the mixture was homogenized. Serial dilution was prepared by transferring 1 mL of the homogenate into 9 mL of BPW to obtain a 10^{-1} dilution. Subsequent serial dilutions were prepared using the same procedure up to 10^{-6}.

Total microbial colonies were enumerated using the pour plate method. A 1 mL aliquot from each dilution was inoculated in duplicate into sterile Petri dishes, followed by the addition of 15–20 mL of PCA medium. Enterobacteriaceae colonies were enumerated using the spread plate method. A 1 mL aliquot from each dilution was distributed in duplicate onto VRBGA plates using three Petri dishes, with volumes of 0.3 mL, 0.3 mL, and 0.4 mL, respectively. The suspension was spread evenly using a sterile spreader. All plates were incubated at 37°C for 24 hours. After incubation, colonies growing on PCA and VRBGA media were counted using a colony counter.

Isolation and Purification of Presumptive *Enterobacter*

Presumptive *Enterobacter* colonies on VRBGA medium were identified based on their purplish-red color, precipitation zone, and mucoid appearance. Three colonies from each sample showing characteristics consistent with presumptive *Enterobacter* were selected and inoculated onto MacConkey agar using the four-quadrant streak plate technique. The plates were incubated at 37°C for 24 hours. Presumptive *Enterobacter* colonies on MacConkey agar appeared pink and mucoid. The selected colonies were then subcultured onto NA medium and re-incubated at 37°C for 24 hours to obtain purified isolates.

Gram Staining and Biochemical Tests

For Gram staining, a single colony from NA medium was aseptically picked and smeared onto a microscope slide. The smear was fixed by passing the slide over a Bunsen flame three times. The slide was stained with crystal violet for 1 minute and rinsed with running distilled water. Lugol's solution was then applied for 1–2 minutes, followed by rinsing with running distilled water. The smear was decolorized using 96% ethanol for 5 seconds and rinsed again with distilled water. Finally, the smear was stained with safranin for 1 minute, rinsed with distilled water, and observed under a microscope.

For the indole, H_2S, and motility tests, a single colony from NA medium was inoculated into 5 mL of SIM medium and incubated at 37°C for 24 hours. After incubation, 3–5 drops of Kovac's reagent were added to detect indole production. For the MR-VP test, one loopful of bacterial culture was inoculated into MR-VP broth and incubated at 37°C for 24 hours. After incubation, five drops of methyl red were added for the methyl red test. For the Voges-Proskauer test, 0.6 mL of α-naphthol and 0.2 mL of 40% KOH were added to the culture, homogenized, and allowed to stand for 2–4 hours. For the citrate test, a colony was streaked onto slanted SCA medium and incubated at 37°C for 24 hours. For the urease test, a single colony was streaked onto slanted urea agar and incubated at 37°C for 24 hours.

Data Analysis

Total microbial and Enterobacteriaceae counts were calculated from two serial dilutions that produced 30–300 colonies. Colony enumeration followed the procedure

specified in ISO 7218. The total microbial count was calculated using the following formula (Suharman et al., 2023):

$$N = \frac{\sum C}{[(1\ x\ n1)+(0{,}1\ x\ n2)]\ x\ (d)}$$

Note:
N = Colony count, expressed as colonies per milliliter or colonies per gram
∑C = The total number of colonies counted on all plates
n1 = The number of tubes in the first dilution that were counted
n2 = The number of colonies counted in the second dilution
d = The first dilution calculated

The TPC value for Enterobacteriaceae was calculated using the following formula (Mayanti et al., 2024):

$$N = \frac{Colony\ count\ x\ \frac{1}{DF}}{V}$$

Note:
N = Colony count, expressed as colonies per milliliter or colonies per gram
DF = Dilution factor
V = Dilution volume

The colony counts were compared with the microbiological limits specified in SNI 9159:2023. Gram staining and biochemical test results were analyzed to support the presumptive identification of *Enterobacter*. All data were analyzed descriptively to determine the microbiological condition of beef liver obtained from traditional markets in Sidoarjo City.

RESULTS AND DISCUSSION

Total Microbial and Enterobacteriaceae Counts

As shown in Table 1, total plate count (TPC) values were determined for beef liver samples collected from traditional markets in Sidoarjo City. Among samples obtained from Larangan Market, two of five samples met the SNI 9159:2023 standard for total microbial contamination, namely sample A2, with a TPC value of 6.0 × 10^5 CFU/g, and sample A5, with a value of 5.65 × 10^5 CFU/g. In contrast, among samples from Porong Market, only one sample met the standard, namely sample B1, with a TPC value of 6.45 × 10^5 CFU/g.

For Enterobacteriaceae counts, none of the beef liver samples met the SNI 9159:2023 standard. Most samples showed total microbial TPC values exceeding 1 × 10^6 CFU/g and Enterobacteriaceae counts exceeding 1 × 10^2 CFU/g. These findings indicate that most beef liver samples exceeded the microbiological limits established in SNI 9159:2023 for foods of animal origin.

Table 1. Total Plate Count (TPC) mean values for beef liver samples from traditional markets in Sidoarjo City

Location	Sample Code	TPC Mean Values of Total Microbial (CFU/g)	Quality Status	TPC Mean Values of Enterobacteriaceae (CFU/g)	Quality Status
Larangan Market	A1	3.64 × 10^6	Exceeds SNI Standard	1.01 × 10^5	Exceeds SNI Standard
	A2	6.0 × 10^5	Meets SNI Standard	6.9 × 10^4	Exceeds SNI Standard

Location	Sample Code	TPC Mean Values of Total Microbial (CFU/g)	Quality Status	TPC Mean Values of Enterobacteriaceae (CFU/g)	Quality Status
	A3	2.7×10^6	Exceeds SNI Standard	4.44×10^5	Exceeds SNI Standard
	A4	4.9×10^6	Exceeds SNI Standard	5.41×10^5	Exceeds SNI Standard
	A5	5.65×10^5	Meets SNI Standard	2.21×10^5	Exceeds SNI Standard
	Mean	2.48×10^6		3.99×10^5	
	Standard Deviation	1.90×10^6		2.38×10^5	
Porong Market	B1	6.45×10^5	Meets SNI Standard	8.8×10^4	Exceeds SNI Standard
	B2	2.23×10^6	Exceeds SNI Standard	2.21×10^5	Exceeds SNI Standard
	B3	3.05×10^6	Exceeds SNI Standard	2.32×10^5	Exceeds SNI Standard
	B4	1.4×10^6	Exceeds SNI Standard	3.9×10^5	Exceeds SNI Standard
	B5	4.45×10^6	Exceeds SNI Standard	4.73×10^5	Exceeds SNI Standard
	Mean	2.36×10^6		2.81×10^5	
	Standard Deviation	1.48×10^6		1.52×10^5	

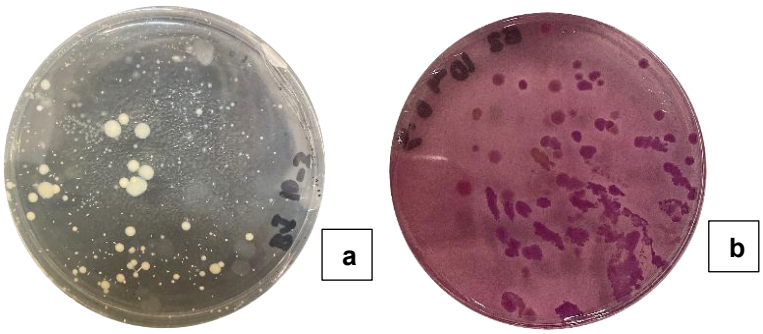

Figure 1. Microbial colony morphology in beef liver samples from traditional markets in Sidoarjo City: (a) Total microbial colonies on PCA medium; (b) Enterobacteriaceae colonies on VRBGA medium

Overall, beef liver samples from Larangan Market showed slightly higher microbial contamination than those from Porong Market, with a mean total microbial TPC of 2.48×10^6 CFU/g and a mean Enterobacteriaceae count of 3.99×10^5 CFU/g. As shown in Figure 4a, the meat stall area at Larangan Market appeared less hygienic than that at Porong Market. The surrounding area contained scattered waste, flies, mud residues on the floor, and visible animal blood.

During sampling, Larangan Market was also more crowded than Porong Market, which may have contributed to less favorable environmental conditions around the meat stalls. Temperature and humidity are known to influence microbial abundance and growth rate (Purnowo et al., 2024). Such conditions may increase the risk of cross-contamination of beef liver from the market environment, equipment, sellers, and consumers. These factors may explain the relatively higher TPC values observed in beef liver samples from Larangan Market compared with those from Porong Market.

Presumptive Identification of Enterobacter Using Biochemical Tests

Enterobacter is a genus within the Enterobacteriaceae family that may contribute to spoilage in animal-derived food products and is commonly used as an indicator of food and beverage safety (Aufani et al., 2023; Höll et al., 2016). Enterobacter spp. may also cause various clinical infections, including bloodstream infections. Wesevich et al. (2020) reported that *E. cloacae* caused bloodstream infections, or bacteremia, in 104 of 150 patients, representing 69% of cases, followed by *E. aerogenes* in 46 of 150 patients, representing 31% of cases. Antibiotic resistance in Enterobacter remains a serious concern because it can complicate clinical treatment.

Colonies suspected to be Enterobacter on VRBGA medium were subsequently isolated on MacConkey agar. On VRBGA medium, presumptive Enterobacter colonies appeared purplish-red, mucoid, and surrounded by a pink precipitation zone. On MacConkey agar, the colonies appeared pink and mucoid (Figure 2). However, phenotypic observation on MacConkey agar alone may be insufficient because this medium is not selective for Enterobacter. Therefore, further biochemical testing was required to support presumptive identification.

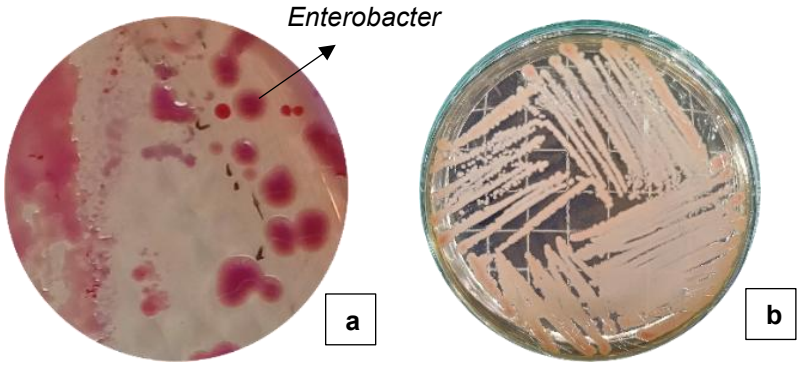

Figure 2. (a) Isolate B2 on VRBGA medium; (b) Isolate B2 on MacConkey medium

Presumptive identification of Enterobacter was based on the biochemical identification criteria for Enterobacteriaceae established by Farmer et al. (1985). As shown in Table 2, isolate B2, which was obtained from a beef liver sample collected at Porong Market, was presumptively identified as *Enterobacter cloacae*. Gram staining showed red, short rod-shaped cells, indicating Gram-negative bacteria. Biochemical testing showed that isolate B2 was indole negative, H_2S negative, motility positive, MR negative, VP positive, citrate positive, and urea positive.

Isolates B1, B4, and B5 showed biochemical characteristics that were similar to those of isolate B2. However, these isolates produced positive MR test results and were therefore not classified as Enterobacter. Other isolates showed biochemical profiles suggestive of other genera within the Enterobacteriaceae family.

The characteristic biochemical profile of Enterobacter generally includes MR-negative, VP-positive, citrate-positive, and variable urease reactions. In the MR test, Enterobacter typically exhibits weak mixed-acid metabolism, resulting in a pH above 4.4 and a yellow color after the addition of methyl red (Aryal, 2022). In the VP test, Enterobacter produces a positive reaction, indicated by an eosin-pink color after the addition of alpha-naphthol and 40% KOH (Aryal, 2018). This reaction occurs because Enterobacter tends to metabolize glucose through the butanediol pathway, producing more acetoin than mixed acids (Vivijs et al., 2014).

In the citrate test, Enterobacter produces a positive result, indicated by a color change of the medium from green to blue. Enterobacter can use citrate as a carbon

source because it possesses citrate synthase and citrate permease enzymes. Citrate metabolism produces ammonia, which increases the alkalinity of the medium and causes the bromothymol blue indicator to change from green to blue (Ulfa et al., 2016).

In the urease test, Enterobacter may produce variable results. A positive reaction is indicated by a pink color change due to ammonia production, whereas a negative reaction is indicated by a yellow color (Prasetya et al., 2019). Some Enterobacter species are generally urease-negative, such as *E. aerogenes*, whereas others, including *E. cloacae*, may produce positive results due to urease enzyme activity (Bhattacharya et al., 2018). Therefore, the biochemical profile of isolate B2 supports its presumptive identification as *Enterobacter cloacae*.

Table 2. Results of Gram staining and biochemical tests on beef liver isolates from traditional markets in Sidoarjo City

Sample Code	Color	Shape	Indole	Mot	H_2S	MR	VP	Citrate	Urea	Presumptive Colony
A1	Red	Rod	(+)	(-)	(-)	(-)	(-)	(-)	(+)	NE
A2	Red	Rod	(+)	(-)	(-)	(+)	(-)	(-)	(+)	NE
A3	Red	Rod	(+)	(+)	(-)	(+)	(-)	(-)	(-)	NE
A4	Red	Rod	(-)	(+)	(-)	(-)	(-)	(-)	(+)	NE
A5	Red	Rod	(+)	(+)	(-)	(+)	(-)	(-)	(-)	NE
B1	Red	Rod	(+)	(+)	(-)	(+)	(+)	(+)	(+)	NE
B2	Red	Rod	(-)	(+)	(-)	(-)	(+)	(+)	(+)	E
B3	Red	Rod	(+)	(+)	(-)	(+)	(-)	(-)	(-)	NE
B4	Red	Rod	(-)	(+)	(-)	(+)	(+)	(+)	(+)	NE
B5	Red	Rod	(-)	(+)	(-)	(+)	(+)	(+)	(+)	NE

Note: Mot = Motility, MR = Methyl Red, VP = Voges-Proskauer, NE = Not Enterobacter, E = Enterobacter

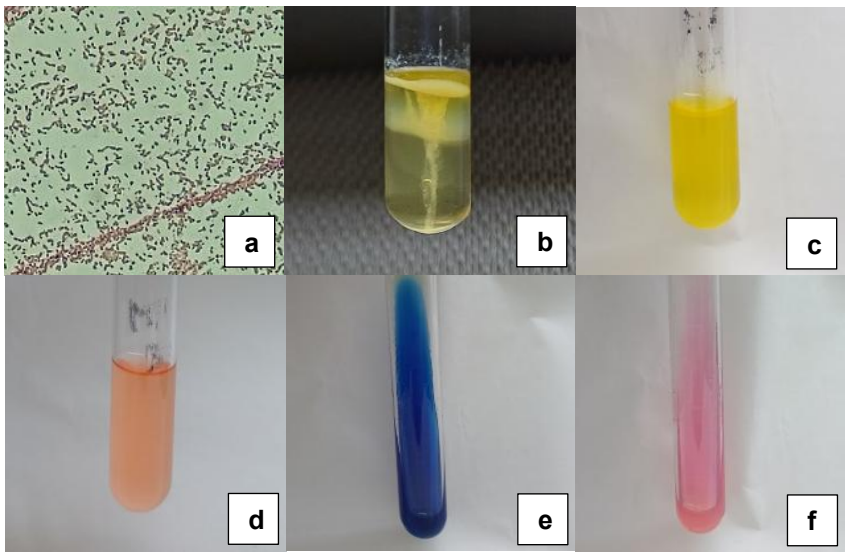

Figure 3. Results of Gram staining and biochemical tests on isolate B2: (a) Microscopic appearance of Enterobacter colonies at 40× magnification; (b) Results of the indole, H_2S, and motility tests; (c) Results of the MR test; (d) Results of the VP test; (e) Results of the citrate test; (f) Results of the urea test

Enterobacter was detected only in isolate B2 from Porong Market, even though samples from this market had a lower mean Enterobacteriaceae count than those from Larangan Market. Based on biochemical testing, isolates from Larangan Market samples (A1–A5) did not show characteristics consistent with Enterobacter. This was particularly evident from the VP and citrate tests, which were negative for all Larangan Market isolates. The biochemical profiles of isolates other than B2 suggested that they may belong to other genera within the Enterobacteriaceae family.

As shown in Figure 4d, some beef liver samples from Porong Market showed early signs of spoilage, indicated by a pale appearance. This discoloration may be associated with myoglobin denaturation (Izzah et al., 2024). Myoglobin denaturation can be influenced by storage duration, temperature, and increased activity of spoilage microorganisms in animal-derived products, including Enterobacter (Hartanto et al., 2023; Höll et al., 2016). Therefore, the presumptive detection of Enterobacter in isolate B2 may be associated with the reduced freshness observed in some beef liver samples from Porong Market.

Environmental Conditions at Traditional Markets in Sidoarjo City

Observations of the meat stall surroundings at both markets are presented in Figure 4. The areas around the meat stalls appeared unhygienic, with scattered waste and visible bloodstains. Beef liver samples from Larangan Market generally appeared fresh, with a brownish-red color (Figure 4b). In contrast, some beef liver samples from Porong Market appeared pale, indicating early signs of spoilage (Figure 4d).

Figure 4. (a) A view of the surroundings of the meat stalls at Larangan Market; (b) A photo of beef liver at Larangan Market; (c) A view of the surroundings of the meat stalls at Porong Market; (d) A photo of beef liver at Porong Market.

Traditional markets may serve as sources of microbial contamination because they are generally open-air environments and often lack adequate refrigeration facilities (Hartanto et al., 2023). In addition to contamination from the market environment, pathogenic microorganisms may already be present in beef liver before slaughter. Enterobacter contamination may originate from unhygienic farm environments, for example through fecal contamination of feed and drinking water consumed by cattle (Bako et al., 2024). Fecal contamination of feed and water can expose cattle to various pathogenic microorganisms, including members of the Enterobacteriaceae family (Gunawan et al., 2022).

Microbial contamination of beef liver may also occur during slaughtering, handling, and distribution. Improper slaughtering procedures can allow microorganisms from the digestive tract to contaminate carcasses or offal, including the liver, which is anatomically located near the digestive tract (Guterres et al., 2025). The absence of cooling treatment after slaughter may further increase microbial growth, particularly during distribution from slaughterhouses to markets (Rani et al., 2023).

The high total microbial and Enterobacteriaceae counts, together with the presumptive detection of Enterobacter, indicate that beef liver samples from Larangan Market and Porong Market did not meet the microbiological safety criteria established in SNI 9159:2023. To obtain the nutritional benefits of beef liver while minimizing the risk of microbial infection, consumers should avoid beef liver that shows signs of reduced freshness. For long-term storage, beef liver should be kept frozen at -20°C to inhibit microbial activity (Afrita et al., 2022). Beef liver is also not recommended for raw consumption and should be cooked at a minimum temperature of 70°C to reduce or inactivate vegetative bacterial cells (Jung et al., 2019).

CONCLUSION

Based on the results of this study, most beef liver samples obtained from traditional markets in Sidoarjo City showed microbial contamination levels that exceeded the microbiological limits established in SNI 9159:2023. Seven out of ten samples had total microbial counts above the maximum permitted limit of ≤1 × 10^6 CFU/g, while all ten samples exceeded the Enterobacteriaceae limit of ≤1 × 10^2 CFU/g. These findings indicate that the microbiological quality of the tested beef liver samples did not fully comply with the required safety standards for animal-derived food products. Furthermore, isolate B2 from Porong Market was presumptively identified as Enterobacter cloacae based on its biochemical profile, including negative indole, H_2S, and methyl red reactions, and positive motility, Voges–Proskauer, citrate, and urease reactions. Overall, the results highlight the need for improved hygiene practices, proper handling, and temperature control during the distribution and sale of beef liver in traditional markets.

RECOMMENDATION

Biochemical testing remains a conventional approach for identifying Enterobacter and is insufficient for definitive species-level confirmation. Therefore, further confirmation using molecular methods, such as PCR, 16S rRNA sequencing, or standardized bacterial identification kits, is necessary to obtain more accurate results. Future studies should also investigate the source of contamination, whether originating from the market environment or from earlier pre-sale stages. In addition, temperature and humidity should be monitored from the slaughterhouse through distribution to the market to better understand their potential contribution to microbial contamination.

ACKNOWLEDGMENT

The author would like to express sincere gratitude to the academic advisor for the assistance and guidance provided throughout the research process and manuscript preparation. The author also thanks the management of Larangan Market and Porong Market in Sidoarjo City for granting permission and access during the sampling process.

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