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Isolation and Identification of Coliform Bacteria in Street Food Products from the Pangandaran Coastal Area, Indonesia

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ABSTRACT

Background: The Pangandaran coastal tourism area, characterized by high visitor density, presents a significant risk for foodborne illness. Data from the West Java Provincial Health Office recorded diarrheal cases in Pangandaran Regency between 2016 and 2024 ranging from 8,003 to 11,789 cases per year, indicating persistent concerns regarding sanitation and food quality.
Aims: This study aimed to evaluate the presence of coliform bacteria in street foods sold in the Pangandaran coastal tourism area through a comprehensive laboratory-based approach.
Methods: Food samples were collected using purposive sampling. Analysis was performed through Total Coliform Count (TCC), coliform confirmation testing, Gram staining, and the IMViC test series.
Results: TCC values in *cilok* (107×10^2 CFU/g) and *pecel sayur* ($2{,}173 \times 10^2$ CFU/g) exceeded the established maximum limits. In contrast, sosis (47×10^2 CFU/g), *siomay* (35×10^2 CFU/g), and *rujak buah* (18×10^2 CFU/g) remained within acceptable limits. Bakwan, cakwe, cimol, and ketan kukus showed no colony growth. Gram staining, coliform confirmation, and IMViC tests confirmed the presence of Gram-negative bacteria in contaminated samples, with characteristics indicative of Escherichia coli based on morphological and biochemical profiles.
Conclusion: *Cilok* and *pecel sayur* contained coliform contamination exceeding the maximum permissible limits, with isolates indicative of E. coli in several samples. Improvements in hygiene, sanitation, and food safety monitoring for street food vendors in the area are strongly recommended, alongside molecular confirmation for definitive species identification.

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1. Introduction

Food safety is a fundamental aspect of public health protection, particularly in developing countries (Amran, 2023). Street food—ready-to-eat products widely consumed across diverse population groups—represents a common dietary staple (Ainutajriani *et al.,* 2023). Inadequate hygiene practices during production, distribution, and serving frequently lead to microbial and chemical contamination, posing risks of foodborne illness including food poisoning and diarrhea (Rangkuti *et al.,* 2021; Siwi & Mogea, 2022).

According to the Indonesian Ministry of Health (2022), diarrhea ranks among the leading causes of mortality, accounting for 14.5% of total national deaths (Zaenab & Azizah, 2025). The consumption of unhygienic street foods in public settings carries a substantial risk of diarrheal disease through microbial contamination, underscoring the critical role of sanitation and hygiene in ensuring food safety (Hutasoit, 2020).

Pangandaran Regency, situated in the southern part of West Java Province, is a designated national strategic tourism area. The region encompasses diverse natural attractions, including nature preserves and river tourism, with Pangandaran Beach serving as its foremost tourist destination (Ashuri & Kustiasih, 2020). As a heavily visited site, Pangandaran Beach sustains intensive food and beverage trading activities. Between 2016 and 2020, domestic tourist arrivals ranged from 1.3 to 2.7 million per year, while international arrivals ranged from 62 to 3,804 per year (Open Data Pangandaran, 2023). This high visitor mobility increases social interaction and the consumption of openly sold ready-to-eat foods, thereby elevating the risk of foodborne contamination associated with poor hygiene and sanitation practices among food handlers (Zaenab & Azizah, 2020).

Data from the Regional Health office, as reported in Open Data Jabar (2025), recorded diarrheal cases in Pangandaran Regency between 2016 and 2024 ranging from 8,003 to 11,789 cases annually. This pattern indicates that diarrhea is a persistent public health concern in the region and may serve as an important indicator of deficiencies in sanitation and food safety, particularly in relation to street food vending in tourist areas (Open Data Jabar, 2025). Proper food handling is essential to produce food that is safe for consumption, consistent with Indonesian Minister of Health Regulation No. 1096/MENKES/PER/VI/2011, which stipulates that food processing facilities must be adequate to ensure operational efficiency, prevent contamination, and facilitate cleaning (Siwi & Mogea, 2022). Failure to maintain adequate hygiene increases the risk of bacterial contamination and the associated burden of foodborne disease (Sadomo & Siwiendrayanti, 2023).

Coliform bacteria are microorganisms commonly used as indicators of microbiological contamination in water and food; their presence signals potential contamination resulting from inadequate sanitation and is associated with illnesses such as diarrhea (Kita *et al.,* 2020; Suryani *et al.,* 2021). This bacterial group comprises several genera within the family Enterobacteriaceae, characterized as Gram-negative, non-spore-forming, rod-shaped organisms capable of growth under both aerobic and anaerobic conditions (Tama *et al.,* 2023). Coliform bacteria originate from fecal contamination by warm-blooded animals; their presence in food or water therefore indicative of poor environmental hygiene and sanitation (Some *et al.,* 2021). Street foods contaminated with coliform bacteria such as *E. coli*, suggest fecal contamination during processing or serving, which may occur via hands, water, the surrounding environment, or insects such as flies (Yulistiani *et al.,* 2023). Coliforms are recognized as mandatory parameters in the microbiological quality evaluation of food and beverages under various national and international standards, including the Indonesian National Standard (SNI) (Febriyossa & Rhamdani, 2024). Consequently, the isolation and identification of coliform bacteria in street foods within tourist areas is essential for contamination risk prevention and food safety assurance.

Previous studies have investigated the presence of coliform bacteria in street foods as indicators of food sanitation quality. Yulistiani *et al.* (2023) reported that all food samples (100%) and the majority

of street food samples (66.6%) examined contained coliform bacteria exceeding permissible limits. A study conducted along Sutorejo Street, Surabaya, found that 75% of street food samples were contaminated with coliforms, comprising 50% *E. coli*, 17%, *Klebsiella* sp., and 8% *Enterobacter* sp. (Ainutajriani *et al.,* 2023). *These novelties confirm that coliform contamination remains a significant concern in commercially sold street foods, reflecting low sanitation standards during food preparation and serving*.

Research on coliform contamination in street foods has been conducted across various settings, from school environments to traditional markets. However, comparable studies in tourist areas such as Pangandaran Beach remain scarce, despite the area's inherent vulnerability to food contamination due to high visitor density and limited sanitation oversight. Furthermore, many existing studies employ restricted methodologies that do not integrate a comprehensive analytical approach encompassing Total Coliform Count (TCC), coliform confirmation testing, and biochemical identification via the IMViC test series. Therefore, a study specifically evaluating the presence of coliform bacteria in street foods at the Pangandaran coastal tourism area using a thorough laboratory-based approach is warranted.

2. Methods

2.1 Food Sample Collection

Street food samples were collected from multiple locations within the Pangandaran coastal tourism area using purposive sampling, with selection criteria based on foods sold in open air settings with potential exposure to environmental contamination. Samples were obtained from two representative locations: (i) roadside vendors; and (ii) beachside vendors. The food types selected as test materials included, *cimol*, *cilok*, *pecel sayur*, *bakwan*, *siomay*, *sosis*, *cakwe*, and *ketan kukus*. Each sample was placed in a ziplock bag and stored in a cool box to maintain cleanliness, preserve microbial viability, and prevent excessive microbial growth. Transport to the laboratory took approximately 30–60 minutes, after which samples were subjected to further analysis to identify potential contamination and other relevant characteristics.

2.2 Bacterial Isolation

Bacterial isolation was performed following the procedure described by Cappucino & Welsh (2019) with modifications. Serial dilutions were prepared at a ratio 1:9 up to a dilution series 10^{-2} using 0.9% physiological saline (NaCl). One gram of each food sample was dissolved in 9 mL of 0.9% physiological saline as the initial dilution (10^{-1}), followed by further dilution to 10^{-2} as required for analysis. The use of 1 g at this stage followed standard microbiological food analysis procedures that enable accurate serial dilution (ISO 6887-1, 2017; Puspitasari *et al.,* 2026). Suspensions were homogenized prior to inoculation to ensure uniform distribution of microorganisms. A 0.1 mL aliquot from each dilution level was inoculated onto Eosin Methylene Blue Agar (EMBA), a selective-differential medium, using the spread plate method to detect *E. coli* and other coliform bacteria. Plates were incubated aerobically at 37°C for 24 hours. Colonies selected for further analysis were those exhibiting a circular morphology of approximately 2–3 mm in diameter, a convex elevation, and a smooth surface on fresh isolation–through surface texture may become rough or mucoid upon repeated subculture. The defining characteristics was the presence of a metallic green sheen on the colony surface (Basavaraju & Gunashree, 2022).

2.3 Colony Identification

Identification of isolated colonies was initiated through macroscopic examination of colony morphology for each dilution sample, including shape, margin, elevation, size, surface, appearance, optical properties, texture, and pigmentation. This observation aimed to characterize the macroscopic features of *E. coli* colonies for subsequent species-level analysis.

2.4 Coliform Colony Enumeration

Bacterial colony counts were determined using the Total Coliform Count (TCC) method. TCC was used to estimate the coliform population from each isolated dilution sample on EMBA. Counts were performed according to standard enumeration criteria, in which valid colony counts fall within the range of 30–300 colonies per plate (Cappucino & Welsh, 2019; Abu-Sini *et al.,* 2023). TCC values were calculated using the following formula (Cappucino & Welsh, 2019):

$$\textbf{\textit{Colony Forming Units}/g} = \frac{mean\ colony\ count}{inoculum\ volume\ x\ dilution\ factor} \qquad (1)$$

2.5 Isolate Purification

Prior to coliform confirmation and biochemical testing, colonies exhibiting characteristic *E. coli* features on EMBA plates were purified through repeated subculture onto fresh EMBA. One loopful of each target colony was inoculated onto EMBA using the streak plate method to obtain single colonies, followed by incubation at 37°C for 24 hours. Subculturing was repeated until a pure culture was confirmed, identified by the presence of metallic green colonies with uniform morphology. The resulting pure cultures were subsequently used for Gram staining, coliform confirmation testing, and the IMViC test series.

2.6 Gram Staining

Gram staining was performed following the procedure of Cappucino & Welsh (2019) to examine the microscopic morphological characteristics of the isolated bacteria. A bacterial smear was prepared on a glass slide pre-cleaned with 70% alcohol. A drop of 0.9% physiological saline was placed on the slide, and one loopful of bacterial isolate was mixed and spread evenly. The smear was air-dried and heat-fixed three times over a Bunsen flame. Crystal violet was applied for 1 minute, followed by rinsing with distilled water and air-drying. Lugol's iodine solution was the applied for 1 minute, followed by rinsing and drying. Decolorization was performed with 95% alcohol for 2 seconds, followed by rinsing with distilled water and drying. Counterstaining was carried out with basic fuchsin for 30 seconds, followed by rinsing and drying. Immersion oil was applied and slides were examined under a microscope at 1,000x magnification.

2.7 Coliform Confirmation Test

The coliform confirmation test was conducted to qualitatively detect coliform bacteria and confirm the presence of *E. coli* in pure cultures purified on EMBA. The procedure was adapted from the Most Probable Number (MPN) method described by Cappuccino & Welsh (2019) with modifications and consisted of two sequential stages: (i) presumptive test; and (ii) confirmed test. In the presumptive test, one loopful of isolate from each dilution sample was inoculated into a test tube containing 9 mL of Lactose Broth (LB) with a Durham tube and incubated at 37°C for 24 hours. Gas bubble formation within the Durham tube was indicative of coliform presence. The confirmed test was subsequently performed to verify that the coliform bacteria detected in the presumptive test were *E. coli*, by transferring positive presumptive cultures into test tubes containing *E. coli* broth (ECB) with Durham tubes, followed by incubation at 37°C for 24 hours. A positive result was indicated by turbidity of the medium and gas bubble formation within the Durham tube.

2.8 IMViC Test

The IMViC test was performed following the procedure of Cappuccino & Welsh (2019) with modifications, using pure cultures purified on EMBA. This test series was conducted to differentiate among microorganisms within the family Enterobacteriaceae and comprised four tests: the Indole test, Methyl Red (MR) test, Voges-Proskauer (VP) test, and Citrate test. The Indole test was performed by

inoculating bacteria into tryptone broth and incubating at 37°C for 24 hours, after which Kovac's reagent was added to detect indole production; a positive result was indicated by the formation of a red ring at the surface of the medium. The Methyl Red test was conducted by incubating bacteria in MR-VP broth, followed by addition of methyl red reagent; a positive result was indicated by a red coloration of the medium. The Voges-Proskauer test was performed by incubating bacteria in MR-VP broth, followed by addition of α-naphthol and KOH reagents; a positive result was indicated by the development of a red color in the medium. The Citrate test was performed by inoculating bacteria onto Simmons Citrate Agar and incubating at 37°C for 24 hours; a positive result was indicated by a color change from green to blue due to an increase in medium pH.

3. Results and Discussion

3.1 Macroscopic Identification of Bacteria

Examination of Eosin Methylene Blue Agar (EMBA) plates inoculated with isolates from nine street food samples revealed considerable variation in colony morphology across samples. Colony growth is illustrated in Figure 1, with six distinct colony types identified across *sosis*, *siomay*, *cilok*, *rujak buah*, and *pecel sayur* samples.

Figure 1. Colony morphology of bacterial isolates from street food samples at Pangandaran Beach on EMBA: (A) *Cilok*, (B) *Sosis*, (C) *Siomay*, (D) *Bakwan*, (E) *Cakwe*, (F) *Rujak*, (G) *Ketan kukus*, (H) *Pecel Sayur*, (I) *Cimol*. (1) 10^{-1} Dilution, (2) 10^{-2} Dilution.

Colony type 1, observed in *sosis*, *siomay*, *rujak buah*, and *pecel sayur*, was circular with an entire margin, convex elevation, small size, and a black coloration with a metallic sheen. Colony type 2, found in *sosis*, *siomay*, and *cilok*, was circular with an entire margin, convex elevation, small size, and a creamy-yellow pigmentation. Colony type 3, present in *sosis*, *siomay*, and *rujak buah*, was circular with an entire margin, convex elevation, punctiform to small size, and purple pigmentation. Colony type 4, observed exclusively in *rujak buah*, was circular with an entire margin, convex elevation, small size, and

a milky-white appearance. Colony type 5, also from *rujak buah*, exhibited a filamentous form with filamentous margins, flat elevation, small size, and dark purple pigmentation. Colony type 6, identified in *pecel sayur*, was circular to irregular with an entire margin, flat elevation, small size, and dark purple pigmentation. In contrast, *bakwan*, *cakwe*, *ketan kukus*, and *cimol* showed no colony growth at either dilution level, with plates remaining visually clear following 24 hours of incubation. According to Basavaraju & Gunashree (2022), colony type 1 shares morphological characteristics consistent with *E. coli*; however, confirmatory testing was required for definitive identification.

3.2 Total Coliform Count (TCC)

The colony counts obtained from EMBA plates at each dilution level for all street food samples collected at Pangandaran Beach are presented in Table 1.

Table 1. TCC results of street food samples from Pangandaran Beach on EMBA.

No	Sample	TCC Results (10^2 CFU/g)		Contamination Limit	Description	Reference
		10^{-1} Dilution	10^{-2} Dilution			
1.	*Cimol*	0	0	1×10^4	Does Not Exceed	ISO 4833-1
2.	*Pecel Sayur*	135	2,173	1×10^5	Exceeds	ISO 4833-1
3.	*Ketan Kukus*	0	0	1×10^5	Does Not Exceed	ISO 4833-1
4.	*Cilok*	107	0	1×10^4	Exceeds	ISO 4833-1
5.	*Sosis*	16	47	5×10^4	Does Not Exceed	ISO 4833-1; SNI 2897
6.	*Siomay*	35	0	1×10^5	Does Not Exceed	ISO 4833-1; SNI2332-3
7.	*Rujak Buah*	8	18	5×10^3	Does Not Exceed	ISO 4833-1
8.	*Cakwe*	0	0	1×10^4	Does Not Exceed	ISO 4833-1
9.	*Bakwan*	0	0	1×10^4	Does Not Exceed	ISO 4833-1

As shown in Table 1, considerable variation in bacterial counts was observed across samples. *Cimol*, *ketan kukus*, *cakwe*, *bakwan*, *sosis*, *siomay*, and *rujak buah* yielded colony counts below the established maximum limits at both dilution levels, suggesting adequate food handling practices in accordance with the Indonesian Food and Drug Authority Regulation No. 13 of 2019 on Maximum Limits of Microbial Contamination in Processed Food. In contrast, *pecel sayur* exhibited the highest contamination levels, recording counts of 135×10^2 CFU/g at the 10^{-1} dilution and $2{,}173 \times 10^2$ CFU/g at the 10^{-2} dilution. Notably elevated counts were also recorded for *cilok*, *rujak buah*, *sosis*, and *siomay*.

Comparison of TCC results against permissible contamination thresholds revealed that certain samples exceeded established limits. *Pecel sayur* counts at both dilution levels substantially surpassed the threshold of 1×10^5 CFU/mL as specified by ISO 4833-1, while *cilok* exceeded the limit of 1×10^4

CFU/g. These exceedances are indicative of contributing factors such as suboptimal sanitation, non-hygienic storage conditions, or contamination from environmental sources during food preparation and serving, rendering the affected products unsafe for consumption (Zaenab *et al.*, 2024).

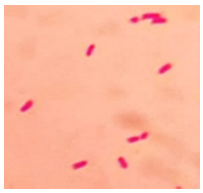
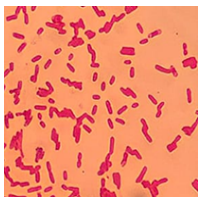
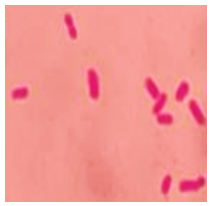
These findings carry direct implications for food safety; particularly concerning foods sold in open-air environments such as beach areas. Elevated microbial contamination increases the risk of foodborne illness, including diarrheal disease and gastrointestinal infections (Sari, 2024). Accordingly, quality control measures are warranted, encompassing improved hygiene standards in food handling, implementation of more stringent sanitation protocols, and targeted education for food vendors on the importance of food hygiene (Sumadewi & Puspaningrum, 2018; Damayanti *et al.,* 2024).

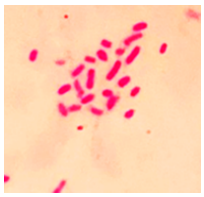
3.3 Gram Staining

Gram staining is a fundamental differential staining technique used to classify bacteria into Gram-positive and Gram-negative groups based on cell wall composition (Yoshimura *et al.,* 2023). The microscopic results obtained from representative colonies grown on EMBA from street food samples collected at Pangandaran Beach are summarized in Table 2.

Microscopic examination of all isolates revealed that colony type 1 from *sosis*, *siomay*, *rujak buah*, and *pecel sayur* consistently exhibited Gram-negative, rod-shaped (bacilli) morphology. These characteristics are consistent with those reported by Chayaningtyas *et al.* (2024), who described *E. coli* as a Gram-negative bacterium appearing red under microscopy with a rod-shaped morphology. To further substantiate species identification, Gram staining results were followed by coliform confirmation testing and the IMViC test series.

Table 2. Microscopic characteristics of Bacterial Isolates on EMBA from street food samples at Pangandaran Beach.

Sample	Microscopic Examination		
	1000x Magnification	Cell Morphology	Gram Type
Sosis		Bacilli	Negative
Siomay		Bacilli	Negative
Rujak		Bacilli	Negative

Pecel		Bacilli	Negative

3.4 Coliform Confirmation Test

Coliform detection in this study was conducted through two sequential stages—the presumptive test and the confirmed test—based on the principles of the Most Probable Number (MPN) method (Uliyanti & Filemon, 2024). It should be noted, however, that the procedure employed did not utilize multiple tube series at each dilution level and was therefore not intended to yield quantitative MPN estimates. Rather, these stages were performed to qualitatively confirm the presence of coliform bacteria based on lactose fermentation and gas production characteristics (Patel *et al.,* 2025; Popescu-Mitroi, 2023). Results for all street food samples tested at Pangandaran Beach are presented in Table 3.

Table 3. Results of coliform detection through presumptive and confirmed tests on bacterial isolates from street food samples at Pangandaran Beach.

Sample	Test		Characteristics
	Presumptive	Confirmed	
Sosis	+	+	Gas production, turbidity
Siomay	+	+	Gas production, turbidity
Rujak	+	+	Gas production, turbidity
Pecel	+	+	Gas production, turbidity

As shown in Table 3, *sosis*, *siomay*, *rujak buah*, and *pecel sayur* yielded positive results in both the presumptive and confirmed tests. In the presumptive test, selected bacterial isolates were inoculated into Lactose Broth and incubated for 24 hours at 35–37°C, after which gas production and turbidity were observed (Hadiansyah *et al.,* 2021). All tested samples produced gas in the Durham tubes, along with acid production and medium turbidity, indicating the presence of coliform bacteria. This is consistent with Amalia *et al.* (2023), who reported that positive presumptive test results are characterized by Durham tube gas formation and medium turbidity resulting from lactose fermentation. Coliform bacteria, as Gram-negative organisms capable of fermenting lactose, produce pyruvic and acetic acids as intermediate metabolites, followed by gas formation and medium turbidity as indicators of bacterial growth and metabolic activity (Nisaa *et al.,* 2020; Hendiana *et al.,* 2022). Although lactose fermentation is characteristic of coliforms, it may also be performed by other microorganisms such as lactic acid bacteria; therefore, positive presumptive results necessitate a confirmed test to verify that the organisms belong to the coliform group (Nengsih *et al.,* 2022).

Positive presumptive results were subsequently carried forward to the confirmed test. *E. coli* Broth (ECB) was used as the confirmatory medium, capable of detecting coliform bacteria when incubated at 37°C and *E. coli* specifically when incubated at 44–45°C (Nengsih *et al.,* 2022). Bacterial suspensions from positive presumptive tubes were transferred to ECB and incubated at 37°C for 24 hours. ECB contains lactose as the carbon source and bile salts as a selective agent that inhibits the growth of Gram-positive and non-enteric bacteria through disruption of cell membranes and protein aggregation within the cytosol (Aji & Fiani, 2021; Nengsih *et al.,* 2022). All samples yielded positive confirmed test results, evidenced by gas production and medium turbidity. According to Hendiana *et al.* (2022), a positive coliform result in the confirmed test is characterized by a change to cloudy yellow turbidity and gas bubble formation within the Durham tube.

Collectively, these results indicate that all tested samples were contaminated with coliform bacteria, serving as indicators of fecal contamination and poor sanitation. The presence of coliforms in food signals the potential for enteropathogenic or toxigenic microbial contamination that poses a public health risk (Sambeka *et al.,* 2024).

3.5 IMViC Test

The IMViC test is an identification method used to characterize bacteria within the family Enterobacteriaceae, particularly for differentiating coliform genera including *E. coli* (Mayanti *et al.,* 2023). In this study, the IMViC test series was applied as a follow-up to the confirmed test to determine the specific genus of the identified coliform isolates. The test comprises four components: the Indole test, Methyl Red (MR) test, Voges-Proskauer (VP) test, and Citrate test (Zuhairiah *et al.,* 2021). Results for all isolates from street food samples at Pangandaran Beach are summarized in Table 4.

As shown in Table 4, all four samples yielded identical IMViC profiles: negative for Indole, VP, and Citrate, and positive for Methyl Red. The Indole test assesses the ability of bacteria to produce indole via tryptophanase enzyme activity, which cleaves tryptophan into indole, pyruvic acid, and ammonia (Zuhairiah *et al.,* 2021). All isolates produced negative Indole results; a positive result would be indicated by a red ring at the medium surface following addition of Kovac's reagent (Latifah & Sofyanita, 2023). The Methyl Red test evaluates the capacity of bacteria to oxidize glucose and produce high-concentration acids as end products (Tuhumury *et al.,* 2022); all samples returned positive results, indicated by a red coloration of the medium, consistent with observations reported by Dewi & Irma (2023). The Voges-Proskauer test detects acetoin production, with a positive result indicated by a red color change following addition of α-naphthol and KOH, while a brownish-yellow or colorless result indicates a negative outcome (Dewi & Irma, 2023). All isolates were VP-negative, indicating that the bacteria were capable of fermenting carbohydrates to produce acid but were unable to generate neutral end-products such as acetoin (Zuhairiah *et al.,* 2021). Finally, the Citrate test assesses the ability of bacteria to utilize citrate as a sole carbon source (Tuhumury *et al.,* 2022); all isolates showed no color change, indicating a negative result, confirming that the isolates could not utilize citrate and therefore failed to raise the medium pH sufficiently to produce a blue coloration (Zuhairiah *et al.,* 2021).

Table 4. IMViC test results for bacterial isolates from street food samples at Pangandaran Beach.

Sample	Results				Suspected Bacterium
	Indole	MR	VP	Citrate	
Sosis	-	+	-	-	*Shigella* sp.
Siomay	-	+	-	-	*Shigella* sp.
Rujak	-	+	-	-	*Shigella* sp.
Pecel	-	+	-	-	*Shigella* sp.
Shigella spp. (Adogaye et al., 2021)	-	+	-	-	
E. coli (Adogaye et al., 2021)	+	+	-	-	

Gram staining of isolates from *sosis*, *siomay*, *rujak buah*, and *pecel sayur* consistently demonstrated Gram-negative, rod-shaped (bacillary) morphology with a red appearance under microscopy, consistent with the microscopic profile of *E. coli* as a Gram-negative organism (Aqil *et al.,* 2025). Macroscopic examination of EMBA plates further revealed metallic green sheen colonies in all four samples—a hallmark of intensive lactose fermentation producing high acid concentrations that precipitate the eosin-

methylene blue complex on the colony surface. Bria *et al.* (2022) similarly reported that *E. coli* on EMBA produces dark purple to black colonies with a characteristic metallic green sheen as a consequence of this precipitation.

Nevertheless, all isolates in this study yielded negative Indole results in the IMViC test, which does not fully conform to the typical biochemical profile of *E. coli*, generally characterized by a positive Indole reaction due to tryptophanase activity on tryptophan (Rifai, 2021). However, phenotypic variation among *E. coli* isolates has been documented in the literature. Antony *et al.* (2016) reported that certain isolates producing metallic green sheen colonies on EMBA exhibited an atypical IMViC profile (− + − −), yet molecular confirmation detected the *uidA* gene in these isolates, supporting their classification as E. coli. These findings indicate that biochemical variation can occur in specific strains and does not preclude identification as *E. coli*.

The negative Indole phenotype observed in morphologically *E. coli*-like isolates may be attributable to several mechanisms. Li & Young (2015) reported that mutations in the tryptophanase gene of *E. coli* can impair phenotypic indole production, yielding a negative result despite confirmed species identity. Additionally, the close phylogenetic relationship between *E. coli* and *Shigella* spp. represents an inherent limitation of conventional biochemical identification (Devanga-Ragupathi *et al.,* 2017). Puspitasari *et al.* (2026), in a comparable study on street food products, similarly reported that colonies displaying metallic green sheen on EMBA remained indicative of *E. coli* despite partial discordance with the typical IMViC biochemical profile.

On the basis of these collective considerations—consistent Gram-negative morphology, strong lactose fermentation characteristics on EMBA, and published evidence supporting IMViC profile variation in specific strains—the isolates in this study are classified as suspected *E. coli*. Nonetheless, molecular confirmation, such as *uidA* gene detection or 16S rRNA gene sequencing, is recommended for definitive species identification.

The presence of suspected *E. coli* in the tested street food samples carries significant food safety implications, particularly for *cilok* and *pecel sayur*, which recorded the highest contamination levels. Elevated contamination in *pecel sayur* may be attributable to prolonged open-air exposure during serving, the use of non-compliant washing water, inadequate storage of raw ingredients, and cross-contamination via utensils and food handler contact (Azzahroh *et al.,* 2021). In the case of *cilok*, although the product undergoes heat treatment during boiling, post-cooking contamination may occur through contact with utensils, sauces, or condiments that do not meet hygienic standards (Siwi & Mogea, 2022). The addition of sauces and condiments after heat treatment represents one of the most commonly reported contamination risk factors in street food safety studies, as this stage involves no further heating that could inactivate contaminants (Kumalasari *et al.,* 2017; Siwi & Mogea, 2022).

These findings underscore that contamination levels are not solely determined by the type of food ingredients used but are substantially influenced by hygiene and sanitation practices throughout handling, processing, and serving. The results therefore not only confirm the presence of fecal indicator bacteria in street foods at Pangandaran Beach but also highlight the urgent need for interventions targeting water source sanitation, food handler hygiene, and cross-contamination control across the open street food supply chain.

4. Conclusion

Analysis of nine street food samples collected from the Pangandaran coastal tourism area confirmed the presence of coliform bacteria in several products, with *cilok* and *pecel sayur* exhibiting bacterial counts exceeding the established maximum permissible limits and therefore deemed unsafe for consumption. Bacterial isolation and identification indicated the presence of suspected *E. coli* in contaminated samples, reflecting poor hygiene and sanitation practices during food processing and serving. These findings underscore the need for improved hygiene and sanitation standards among food

vendors, routine food safety monitoring by relevant authorities, and targeted education for vendors on hygienic food handling and preparation to prevent bacterial contamination in street foods at the Pangandaran coastal tourism area. Furthermore, further investigation employing more specific identification methods—including molecular confirmation—is recommended to definitively characterize the contaminant species and verify the presence of *E. coli* in the implicated samples.

5. Authors Note

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

6. Reference

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