Indonesian Journal of Pharmaceutical Education (e-Journal) 2026; 6 (2): 391-403
ISSN: 2775- 3670 (electronic)
Journal Homepage: http://ejurnal.ung.ac.id/index.php/ijpe/index
DOI: 10.37311/ijpe.v6i2.38414

Coliform and *Escherichia coli* Contamination Ice Products from Cilengkrang Village, Cirebon Regency

Bambang Karsidin[1]*, Siti Pandanwangi TW[1], Arief Rahman Afief[1]
[1] *Faculty of Pharmacy, University YPIB Majalengka, West Java, Indonesia.*
E-mail: bambangkarsidin@lecturer.univypib.ac.id

Article Info:
Received: 23 April 2026
in revised form: 3 June 2026
Accepted: 14 July 2026
Available Online: 1 August 2026

Keywords:
Coliform;
Escherichia coli;
Ice contamination;
Most Probable Number;
Microbiological quality

Corresponding Author:
Bambang Karsidin
Faculty of Pharmacy
University YPIB Majalengka
City
Indonesia
E-mail:
bambangkarsidin@lecturer.univypib.ac.id

ABSTRACT

Ice products including crystal ice and commercially packaged ice cubes are routinely consumed in peri-urban Indonesia, yet freezing does not confer sterility. This descriptive, laboratory-based study examined four ice specimens from Cilengkrang Village, Cirebon Regency, using the three-stage Most Probable Number (MPN) method for coliform enumeration and IMViC–TSIA profiling for *Escherichia coli* identification. All specimens exceeded the regulatory threshold of 0 CFU/100 mL stipulated under Permenkes No. 2/2023, with MPN values ranging from 29 to 1,100/100 mL. Biochemical characterisation was substantially consistent with *E. coli*, however, a positive Simmons Citrate reaction was observed a finding that, in the absence of single colony purification, cannot be attributed exclusively to atypical *E. coli*, as it is equally consistent with the co-presence of citrate-positive Coliform genera such as Klebsiella spp. or Enterobacter spp. within a mixed microbial population. These results constitute preliminary evidence of microbiological risk, warranting expanded sampling and molecular confirmation.

How to cite (APA 6th Style):
Karsidin,B.,Pandanwangi.S.,Afief.,A.R.(2026). *Coliform and Escherichia coli Contamination Ice Products from Cilengkrang Village, Cirebon Regency*. *Indonesian Journal of Pharmaceutical Education (e-Journal)*, *6*(2), 391-403.

ABSTRAK

Produk es termasuk es kristal dan es batu kemasan plastik secara rutin dikonsumsi di komunitas pinggiran perkotaan Indonesia, namun proses pembekuan tidak menjamin sterilitas. Penelitian deskriptif berbasis laboratorium ini memeriksa empat sampel es dari Desa Cilengkrang, Kabupaten Cirebon, menggunakan metode *Most Probable Number* (MPN) tiga tahap untuk enumerasi coliform serta profil IMViC–TSIA untuk identifikasi *Escherichia coli*. Seluruh sampel melampaui ambang batas 0 CFU/100 mL yang ditetapkan Permenkes No. 2/2023, dengan nilai MPN berkisar antara 29 hingga 1.100/100 mL. Karakterisasi biokimia sebagian besar konsisten dengan *E. coli*, namun ditemukan reaksi Simmons Citrate positif yang tanpa pemurnian koloni tunggal tidak dapat secara eksklusif diatribusikan pada *E. coli* atipikal, karena sama konsistennya dengan keberadaan genus coliform citrate positif seperti *Klebsiella* spp. atau *Enterobacter* spp. dalam populasi mikroba campuran. Temuan ini merupakan bukti awal risiko mikrobiologis yang memerlukan pengambilan sampel lebih luas dan konfirmasi molekuler.

Kata Kunci: Coliform; *Escherichia coli*; Kontaminasi es; *Most Probable Number*; Kualitas mikrobiologis

1. Introduction

Ice constitutes an integral component of the food and beverage supply chain in tropical climates, where ambient temperature accelerates microbial proliferation and spoilage of perishable commodities. In Indonesia, crystal ice and packaged ice cubes are routinely used by informal street vendors and small-scale food service establishments, particularly in peri-urban and rural areas with limited refrigeration infrastructure. Ice is often presumed microbiologically safe under the assumption that freezing eliminates contamination; however, this assumption is not supported by microbiological evidence, as freezing does not confer sterilization and pathogenic or indicator organisms may persist within the ice matrix and remain infectious upon thawing or oral contact [1][2].

The presence of Coliform bacteria and *E. coli* in ice is a recognized indicator of fecal contamination originating from source water or post-production handling failures, and represents a microbiological risk of direct public health relevance. Coliform bacteria, as Gram negative, lactose fermenting facultative anaerobes, are conventionally used as surrogate indicators of fecal pollution, correlating with the probable presence of enteric pathogens [1]. *E. coli* holds particular significance both as an indicator organism, owing to its enteric origin in warm blooded animals, and as a pathogen through virulent serotypes, Enterohemorrhagic *E. coli* O157:H7, for example, produces Shiga toxins capable of precipitating haemolytic uraemic syndrome [3]. Enteric illness associated with *E. coli* contaminated food and water has been documented across diverse settings, including Indonesian peri-urban populations where ice containing beverages are implicated in enteric fever transmission [4].

Coliform and *E. coli* contamination has been reported in bulk and packaged ice across diverse settings, frequently exceeding established safety thresholds. In Indonesia, such contamination has been documented in ice cubes sold by informal vendors [5] and in refilled drinking water products [6][2], indicating persistent non-compliance across the supply chain. A systematic review by Dipta et al. identified raw water quality, inadequate facility hygiene, and post-production handling as principal determinants of *E. coli* presence in commercial ice, suggesting that contamination reflects systemic rather than single point deficiencies [7]. Comparable patterns have been reported internationally in low and middle income settings with limited regulatory oversight of informal ice production [8][9].

Microbiological quality of ice for human consumption in Indonesia is governed by Ministry of Health Regulation No. 2 of 2023, which sets a maximum permissible concentration of 0/100 mL for Coliform bacteria and *E. coli* in drinking water and ice [10]. This zero-tolerance standard reflects the position that any detectable fecal indicator organism constitutes a potential exposure risk, with particular relevance for high-risk subpopulations such as children and immunocompromised individuals [11][1]. Despite this regulatory clarity, enforcement at the level of small-scale informal producers remains limited, underscoring the continued need for independent microbiological surveillance.

Empirical data on ice quality specific to peri-urban communities in Cirebon Regency, West Java, remain limited. Cilengkrang Village, in Pasaleman District, procures both crystal ice and packaged ice cubes from informal small-scale producers whose hygienic practices have not been systematically evaluated. The visual distinction between transparent crystal ice and opaque packaged ice cubes is commonly used by consumers as a proxy quality indicator [12], although the reliability of this criterion as a predictor of microbiological compliance has not been validated.

This study was conducted to determine the presence and concentration of Coliform bacteria and *E. coli* in crystal ice and packaged ice cube specimens from Cilengkrang Village, using the three-stage Most Probable Number (MPN) method supplemented by IMViC and Triple Sugar Iron Agar testing for *E. coli* identification. Findings were evaluated against Permenkes No. 2 of 2023, with the aim of generating preliminary evidence on the microbiological safety of locally circulating ice products and identifying probable contamination pathways within the study area.

2. Methods

Study Design

The present study adopted a descriptive, laboratory-based design with a quantitative analytical orientation. Examination was limited to four specimens two crystal ice samples and two plastic packaged ice cube samples each procured from two separate distribution points. This restriction was attributable to the exploratory scope of the investigation and the limited accessibility of production sites at the time of sample collection. Accordingly, the constrained sample size is recognized as a limitation on the generalizability of the findings.

Prior to microbiological analysis, ice specimens were melted under aseptic conditions within the laminar airflow cabinet. Specimen containers were sealed with sterilized aluminum foil, and their exterior surfaces were decontaminated with 70% (v/v) ethanol prior to opening, while all transfer instruments were flame sterilized over a Bunsen burner immediately before use. Melted samples were processed for inoculation without delay to minimize secondary bacterial proliferation or extraneous contamination during handling.

Instruments and Materials

Laboratory instrumentation was organized according to functional application and encompassed apparatus for sterilization and aseptic processing (autoclave, ALP; laminar airflow cabinet), incubation and thermal operations (incubator, Memmert; Bunsen burner; hot plate, Heidolph), and microscopic examination (compound microscope, Olympus; standard glass slides), alongside volumetric transfer devices (micropipette, Transferpette; volumetric pipettes; bulb pipette filler), a gravimetric analytical balance (Ohaus), and ancillary apparatus comprising petri dishes, test tubes,

Durham tubes, inoculating loops, a test tube rack, a vortex mixer, and aluminum foil. Study materials were classified into three functional categories: biological specimens consisting of crystal ice and plastic packaged ice cubes sourced from Cilengkrang Village, East Cirebon; microbiological culture media, namely Nutrient Agar (NA; Oxoid), Lactose Broth (LB; Oxoid), Eosin Methylene Blue Agar (EMBA; Oxoid), Brilliant Green Lactose Bile Broth (BGLB; Oxoid/Merck), Methyl Red–Voges Proskauer (MRVP) broth (Merck), Sulfide Indole Motility (SIM) medium (Merck), Simmons Citrate Agar (Merck), and Triple Sugar Iron Agar (TSIA; Merck), each prepared per the respective manufacturer's specifications [2], and biochemical and staining reagents consisting of crystal violet, Lugol's iodine solution, 70% (v/v) ethanol, safranin, methyl red indicator, Barritt's reagent A (5% alpha-naphthol in ethanol), Barritt's reagent B (40% potassium hydroxide solution), Kovac's reagent, physiological saline (0.9% NaCl), immersion oil, distilled water, double-distilled water, and denatured ethanol [13][14].

Sample Collection

Samples were collected from Cilengkrang Village, Pasaleman District, Cirebon Regency, in August 2025 through purposive sampling. A total of four specimens were obtained two crystal ice samples (X1 and X2) sourced from two separate restaurants, and two plastic packaged ice cube samples (V1 and V2) procured from two small-scale food stall vendors, each specimen weighing 500 grams. Following collection, all specimens were maintained under refrigeration at ±4°C to retard bacterial proliferation and preserve the microbiological integrity of the samples prior to laboratory analysis.

Preparation of Culture Media

Seven culture media were employed in this study: Nutrient Agar (NA) slants, Lactose Broth (LB), Eosin Methylene Blue Agar (EMBA), Sulfide Indole Motility (SIM) medium, Methyl Red–Voges Proskauer (MRVP) broth, Simmons Citrate Agar, and Triple Sugar Iron Agar (TSIA). Each medium was selected on the basis of its established selective or differential properties relevant to the detection and biochemical characterisation of Coliform bacteria and *E. coli* [15]. All media were prepared in accordance with the respective manufacturers' specifications, dissolved in distilled water, and homogenised by continuous agitation using a magnetic stirrer until complete dissolution was achieved. Sterilisation was subsequently performed by moist-heat autoclaving at 121°C and 103.4 kPa (15 psi) for 15 minutes, a parameter set widely recognised as the standard condition for achieving sterility in microbiological growth media [16][17].

Coliform Testing (Most Probable Number Method)

Microbiological enumeration of Coliform bacteria was carried out through the three-stage Most Probable Number (MPN) procedure in accordance with established bacteriological water quality analysis protocols. In the presumptive stage, melted ice samples were aseptically inoculated into Lactose Broth (LB) at volumes of 10 mL, 1 mL, and 0.1 mL in a 3-3-3 dilution series [18][19]. The inoculated tubes were incubated at 37°C for 24–48 hours; a positive reaction was defined by the concurrent occurrence of gas production within the inverted Durham tube and visible turbidity of the medium, both indicative of lactose fermentation. In the confirmatory stage, tubes yielding a positive presumptive result were sub-cultured into Brilliant Green Lactose Bile Broth (BGLB), a selective medium in which bile salts and brilliant green suppress non-coliform flora, and incubated at 37°C for 24 hours [20][2]. Positivity was confirmed by gas production in the Durham tube accompanied by medium turbidity. In the completed stage, positive BGLB cultures were streaked onto Eosin Methylene Blue Agar (EMBA) by the streak plate method. The formation of colonies exhibiting a metallic green sheen

arising from eosin-methylene blue dye precipitation under the acidic microenvironment generated by vigorous lactose fermentation was regarded as indicative of *E. coli*, whereas colonies presenting a pink coloration were considered indicative of non-fecal Coliform organisms [21][22].

Identification of Escherichia coli

Identification of *E. coli* was conducted through Gram staining, biochemical characterisation via the IMViC test battery, and fermentative profiling using Triple Sugar Iron Agar (TSIA). Gram staining employed crystal violet, Lugol's iodine, 95% ethanol, and safranin as successive reagents, with microscopic examination performed under oil immersion at 1,000× magnification. The IMViC comprised the Indole test (SIM medium with Kovac's reagent), the Methyl Red test (MRVP medium with methyl red reagent), the Voges–Proskauer test (MRVP medium with 5% alpha-naphthol and 40% KOH), and the Simmons Citrate test. TSIA was employed to assess the capacity for glucose, lactose, and sucrose fermentation, as well as H_2S production [6][23].

Data Analysis

Escherichia coli concentrations were expressed as Most Probable Number (MPN) values per 100 mL of sample, derived from the 3-3-3 tube series. The MPN index represents a statistically derived estimate of microbial density within a liquid sample, calculated from the pattern of positive and negative fermentation outcomes observed across serially diluted inoculation volumes [1]. Resultant MPN values were evaluated against the regulatory threshold established under Indonesian Ministry of Health Regulation No. 2 of 2023 (Permenkes No. 2/2023), which stipulates a maximum permissible concentration of 0/100 mL for Coliform bacteria and *E. co*li as microbiological quality criteria for drinking water [10]. Specimens yielding MPN values in excess of this threshold were classified as non-compliant with the applicable standard. Given the descriptive and exploratory nature of the study design, no inferential statistical procedures were applied [24].

3. Results and Discussion

Presumptive stage results - Most Probable Number (MPN) method

The presumptive test functions as an initial screening procedure for Coliform bacteria, predicated on their capacity to ferment lactose in Lactose Broth (LB) with concomitant gas production [25]. Positive results were obtained from all four specimens (X1, X2, V1, and V2), as evidenced by gas accumulation within the inverted Durham tubes and visible turbidity of the medium following incubation at 37°C for 24–48 hours. The MPN values for all specimens attained >1,100/100 mL (3-3-3 combination), surpassing the permissible threshold of 0/100 mL stipulated under Permenkes No. 2 of 2023 [10]. The detailed results are presented in **Table 1**.

Table 1. Presumptive stage results

Sample Code	10 mL (positif/3)	1 mL (positif/3)	0.1 mL (positif/3)	Combination tube	MPN/100 mL
X1	3/3	3/3	3/3	3-3-3	>1100
X2	3/3	3/3	3/3	3-3-3	>1100
V1	3/3	3/3	3/3	3-3-3	>1100
V2	3/3	3/3	3/3	3-3-3	>1100

Confirmatory stage results - Most Probable Number (MPN) method

The confirmatory test employed Brilliant Green Lactose Bile Broth (BGLB), a selective medium that inhibits the proliferation of Gram-positive organisms and non-coliform Gram-negative bacteria, thereby restricting growth to genuine Coliform bacteria. A positive result was defined by gas production within the inverted Durham tube accompanied by turbidity of the medium. At this stage, the MPN values of specimens X1 and V1 declined to 240/100 mL, whereas X2 and V2 remained at >1,100/100 mL. The reduction observed in X1 and V1 is attributable to the selective suppression of non-coliform microorganisms by the bile salts and brilliant green constituents of BGLB, such that enumeration was restricted to Gram-negative Coliform organisms [12]. The results are presented in **Table 2**.

Table 2. Confirmatory stage results

Sample Code	10 mL (positif/3)	1 mL (positif/3)	0.1 mL (positif/3)	Combination tube	MPN/100 mL
X1	3/3	3/3	0/3	3-3-0	240
X2	3/3	3/3	3/3	3-3-3	>1100
V1	3/3	3/3	0/3	3-3-0	240
V2	3/3	3/3	3/3	3-3-3	>1100

Completed stage results - Most Probable Number (MPN) method

The completed test was conducted by subculturing positive confirmatory cultures onto Eosin Methylene Blue Agar (EMBA) using the streak plate method. EMBA contains both eosin and methylene blue dyes, which selectively inhibit the growth of Gram-positive bacteria while permitting the propagation of Gram-negative organisms. Isolates of *E. coli*, through vigorous lactose fermentation with consequent acidification of the medium, characteristically produce colonies exhibiting a metallic green sheen on EMBA a differential coloration arising from dye precipitation under acidic conditions [26]. Pink colonies are regarded as indicative of non-fecal Coliform organisms. At this stage, specimens X1 and V1 yielded MPN values of 240/100 mL and 29/100 mL, respectively, whereas specimens X2 and V2 remained at >1,100/100 mL. Each of these values exceeded the regulatory threshold of 0/100 mL stipulated under Permenkes No. 2 of 2023 [10]. The detailed results are presented in **Table 3**.

Table 3. Completed stage results

Sample Code	10 mL (positif/3)	1 mL (positif/3)	0.1 mL (positif/3)	Combination tube	MPN/100 mL
X1	3/3	3/3	0/3	3-3-0	240
X2	3/3	3/3	3/3	3-3-3	>1100
V1	2/3	3/3	0/3	2-3-0	29
V2	3/3	3/3	3/3	3-3-3	>1100

Identification of *Escherichia coli* results (IMViC and TSIA)

The indole test, performed using SIM medium with subsequent addition of Kovac's reagent, yielded negative results for all samples, as the medium retained its original yellowish coloration following the addition of the reagent, with no red/pink ring formed at the medium surface (**Figure 1a**), indicating an absence of detectable tryptophanase activity and, consequently, an absence of indole production by the

isolates, a finding that deviates from the indole-positive profile typically reported for *E. coli* [27].

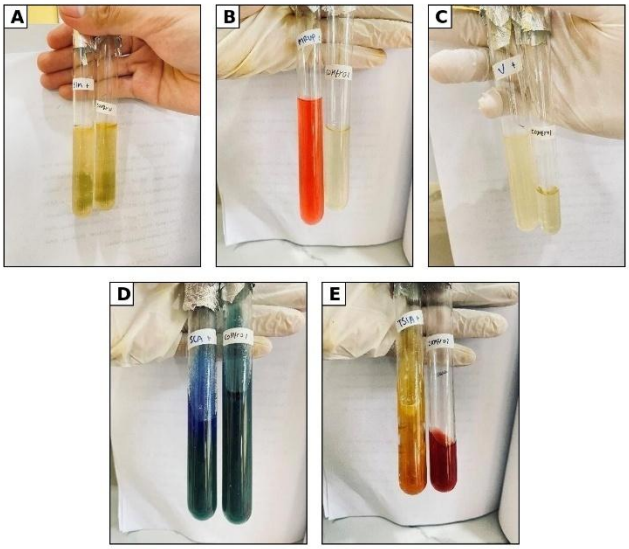

Figure 1. Biochemical identification of the bacterial isolates using the IMViC test battery and Triple Sugar Iron Agar (TSIA).

The Methyl Red test demonstrated positive results across all specimens, as indicated by a distinct red coloration of the MRVP medium following addition of methyl red indicator (**Figure 1b**), consistent with the production of stable mixed-acid fermentation end products during glucose metabolism. The Voges–Proskauer test yielded negative results for all specimens, with no pink coloration observed upon addition of Barritt's reagents (**Figure 1c**), in accordance with the established inability of *E. coli* to produce acetoin as an intermediate of the butanediol fermentation pathway [28].

The Simmons Citrate test yielded positive results for all specimens, as evidenced by a colour change from green to blue in the medium (**Figure 1d**) a finding that deviates from the conventional biochemical profile of *E. coli* and is interpreted in the Discussion section. The TSIA test revealed an acid reaction in both the slant and the butt of the medium across all specimens, as indicated by a uniformly yellow coloration (**Figure 1e**), consistent with fermentation of glucose, lactose, and sucrose. Gas production was evidenced by physical displacement or disruption of the agar column, while the absence of a black precipitate confirmed that hydrogen sulphide (H_2S) was not generated a fermentative profile substantially consistent with *E. coli* [27].

Gram Staining Results

Microscopic examination of colonies isolated on Nutrient Agar, following Gram staining performed at 1,000× magnification under oil immersion, revealed short, rod-shaped bacterial cells with pink staining (**Figure 2**), thereby confirming the Gram-negative character of the isolates. This tinctorial result reflects the cell wall ultrastructure of *E. coli*, in which a thin peptidoglycan layer and an outer lipopolysaccharide membrane permit decolorization by ethanol during the procedure, the subsequent retention of safranin as a counterstain produces the characteristic pink morphology observable under light microscopy [5].

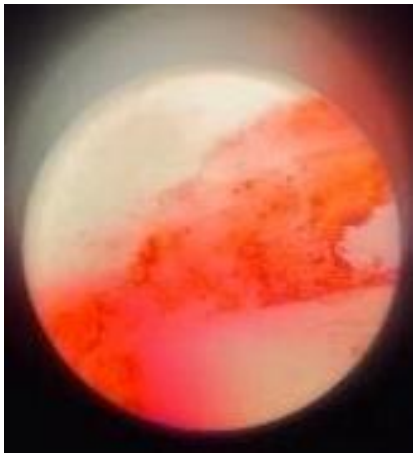

Figure 2. Microscopic morphology of the bacterial isolate revealed by Gram staining technique

Discussion

The detection of coliform and *Escherichia coli* across all four ice specimens, with MPN values ranging from 29/100 mL to >1,100/100 mL, represents a microbiological finding of public health relevance, as each value surpassed the regulatory threshold of 0/100 mL stipulated under Permenkes No. 2 of 2023 [10]. The magnitude and distribution of MPN values across specimens merit systematic interpretation, as they provide indirect evidence regarding the probable contamination pathways operative at the respective production and distribution points.

The marked inter-specimen variability in MPN values X1 yielding 240/100 mL and V1 yielding 29/100 mL, compared with X2 and V2 both exceeding 1,100/100 mL indicates that contamination burden is not uniformly distributed across vendors and is unlikely to derive from a single shared source. This pattern is consistent with the findings of Dipta et al. [7], who identified raw water quality and vendor-specific hygiene and sanitation practices as independent determinants of *E. coli* burden in ice products, and who demonstrated that contamination profiles differ substantially between production facilities within the same geographic locality. Accordingly, specimens yielding MPN values of >1,100/100 mL most probably reflect the use of source water drawn from groundwater sources subject to fecal infiltration, or water subjected to inadequate pretreatment prior to freezing, whereas the comparatively lower values recorded for X1 and V1 may suggest partial attenuation of contamination through elementary processing measures such as preliminary sedimentation or boiling without, however, achieving complete microbiological elimination.

The physical characteristics of the two ice types provide further analytical context. Crystal ice, characterized by optical transparency, is generally associated with water that has undergone degassing through boiling or mechanical aeration a process that removes dissolved gases but does not inherently confer microbiological safety. Plastic packaged ice cubes, by contrast, typically exhibit a whitish or opaque appearance attributable to the retention of dissolved gases in unprocessed or minimally treated water [29]. Although this visual distinction might suggest differential raw water treatment between the two product types, the present findings indicate that optical transparency was not associated with reduced microbiological contamination, as crystal ice specimen X2 yielded MPN values equivalent to those of the most heavily contaminated plastic packaged ice specimen (V2). This dissociation between physical appearance and microbiological quality underscores the inadequacy of organoleptic

assessment as a surrogate indicator of hygienic compliance a conclusion consistent with the observations of Hidayah et al. [12] who reported the absence of a predictable relationship between ice type and Coliform contamination level in specimens from Karawang City.

Post-production contamination represents a mechanistically distinct and comparably plausible pathway, independent of raw water quality. Coliform organisms, including *E. coli*, may be introduced or proliferated at multiple stages along the production and distribution chain through contact with inadequately decontaminated equipment, non-sterile packaging materials, or manual contact by food handlers during serving and distribution. Dipta et al. [7] demonstrated that inadequate personal hygiene among handlers and insufficient sanitation of storage containers were independently associated with *E. coli* presence in ice, irrespective of source water quality, indicating that post-production contamination may be sufficient to render a microbiologically compliant product non-compliant with regulatory standards. The possibility of incidental environmental contamination during laboratory processing, while attenuated by the aseptic procedures employed in this study, cannot be entirely excluded as a confounding variable, and is acknowledged as an inherent limitation of descriptive microbiological investigations conducted outside fully controlled conditions [26].

The biochemical profile obtained through the IMViC and TSIA test battery merits further discussion. The positive methyl red reaction, combined with the negative Voges–Proskauer result and acid-with-gas production on TSIA in the absence of H_2S, is consistent with the established profile of *E. coli* [27]. However, two deviations from this expected pattern were observed in the present study. The first deviation is the indole-negative result, given that this organism is conventionally characterized by constitutive tryptophanase activity and is therefore expected to yield a positive indole reaction. The second deviation is the positive reaction observed in the Simmons Citrate test, confirmed by the development of a blue coloration in the medium. *E. coli* is conventionally characterized as citrate-non-utilizing, as it lacks the membrane transport systems and enzymatic apparatus required to assimilate citrate as a sole carbon source under aerobic conditions [27].

These combined deviations admit two interpretive possibilities: that the isolates represent atypical *E. coli* variants exhibiting reduced tryptophanase expression together with acquired citrate-utilization capacity, phenotypically comparable to atypical or mutant strains documented under experimental selective pressure, or, alternatively, that the specimens harboured mixed microbial populations in which indole-negative, citrate-utilizing Coliform organisms, such as Klebsiella spp. or Enterobacter spp., genera for which an indole-negative, citrate-positive profile constitutes the expected biochemical pattern, were co-present alongside, or were mistakenly identified as, *E. coli*. The inherent limitation of the MPN method in resolving individual species within mixed microbial populations underscores the need for confirmatory molecular identification in subsequent investigations. It should also be acknowledged that colony purification through re-streaking of single colonies was not performed prior to subculturing the isolates onto the IMViC and TSIA media; consequently, the possibility that the indole-negative and citrate-positive results reflect a mixed bacterial population, rather than a genuinely atypical *E. coli* phenotype, cannot be excluded and is regarded as a methodological limitation of the present study.

The public health implications of the contamination levels documented in this study are of clinical and epidemiological concern. Coliform bacteria are recognized indicators of fecal contamination and are associated with the potential presence of a

broader spectrum of enteric pathogens [12]. *E. coli*, while functioning as a commensal organism within the human gastrointestinal tract under physiological conditions, encompasses pathogenic serotypes capable of causing enteric illness following ingestion of contaminated food or water. Enterohemorrhagic *E. coli* (EHEC), in particular, produces Shiga toxin, which disrupts colonic epithelial integrity and may precipitate haemolytic uraemic syndrome a thrombotic microangiopathy characterized by microangiopathic haemolytic anaemia, acute kidney injury, and thrombocytopenia [3]. Vollaard et al. [4] documented an epidemiological association between the consumption of beverages containing contaminated ice and the incidence of enteric fever, including typhoid, in Indonesian urbasn and peri-urban populations a finding pertinent to the food vending context of Cilengkrang Village, where ice is routinely incorporated into commercially served beverages and consumed without further thermal processing.

The findings of this study indicate that both crystal ice and plastic packaged ice cubes distributed in Cilengkrang Village present a microbiological risk attributable to contamination at the level of raw water sourcing, production hygiene, post-production handling, or most probably a combination of these factors operating at differing magnitudes across individual vendors. The convergence of contamination across both ice types, despite their distinct production characteristics, suggests a systemic rather than product-specific deficiency in hygiene and sanitation standards at the local level a conclusion consistent with comparable findings reported from other semi-urban settings in Indonesia [30][31].

A principal methodological limitation of this study pertains to sample size. The analysis of only four specimens restricts the applicability of the findings to the specific distribution points examined and precludes meaningful generalization to the broader ice supply within Cilengkrang Village. Variability in contamination levels across vendors, seasonal periods, and production practices cannot be adequately characterized from such a restricted dataset. The results are therefore best interpreted as preliminary evidence of microbiological risk rather than as a definitive assessment of ice quality across the study area.

4. Conclusion

This study demonstrated that all four ice specimens examined two crystal ice samples (X1, X2) and two plastic packaged ice cube samples (V1, V2) collected from Cilengkrang Village, Pasaleman District, Cirebon Regency were contaminated with Coliform bacteria and Escherichia coli, as evidenced by MPN values ranging from 29/100 mL to >1,100/100 mL, each surpassing the maximum permissible threshold of 0/100 mL stipulated under Indonesian Ministry of Health Regulation No. 2 of 2023. Biochemical characterisation through the IMViC test battery and TSIA profiling yielded results substantially consistent with *E. coli*, notwithstanding an atypical positive Simmons Citrate reaction that warrants further confirmatory investigation, including molecular identification, to resolve whether this finding reflects atypical strain variants or the co-presence of citrate-utilizing Coliform organisms within mixed microbial populations. The convergence of contamination across both ice types, despite their distinct physical and production characteristics, suggests that the observed microbiological non-compliance is attributable to systemic deficiencies in raw water quality, production hygiene, or post-production handling practices or a combination thereof rather than to factors specific to any single product type. Given the restricted sample size of four specimens, the findings are appropriately interpreted as preliminary evidence of microbiological risk at the specific distribution points examined, and

broader generalisation to the wider ice supply within the study area requires validation through investigations employing larger and more representative sampling frames. Future research should therefore prioritize molecular identification, such as 16S rRNA gene sequencing, of the indole-negative, citrate-positive isolates to confirm whether the observed biochemical profile represents an atypical *E. coli* phenotype or a co-occurring Coliform species.

Acknowledgements:
The authors gratefully acknowledge the Faculty of Pharmacy, University YPIB Majalengka, for providing access to laboratory facilities and institutional resources that supported the conduct of this study.

Conflict of Interest:
The authors declare no conflict of interest.

References

[1] S. Some, R. Mondal, D. Mitra, D. Jain, D. Verma, and S. Das, "Microbial pollution of water with special reference to coliform bacteria and their nexus with environment," *Energy Nexus*, vol. 1, art. no. 100008, 2021. [Online]. Available: https://doi.org/10.1016/j.nexus.2021.100008

[2] C. Fatimah, S. Safriana, and S. Andriani, "Coliform contamination test using the MPN method in dug-well water, bore-well water, and municipal drinking water," *Journal of Pharmaceutical and Health Research*, vol. 5, no. 1, pp. 64–72, 2024. [Online]. Available: https://doi.org/10.47065/jharma.v5i1.4955

[3] S. M. Gambushe, O. T. Zishiri, and M. E. El Zowalaty, "Review of *Escherichia coli* O157:H7 prevalence, pathogenicity, heavy metal and antimicrobial resistance: African perspective," *Infection and Drug Resistance*, vol. 15, pp. 4645–4673, 2022. [Online]. Available: https://doi.org/10.2147/IDR.S365269

[4] A. M. Vollaard, "Risk factors for typhoid and paratyphoid fever in Jakarta, Indonesia," *JAMA*, vol. 291, no. 21, pp. 2607–2615, 2004. [Online]. Available: https://doi.org/10.1001/jama.291.21.2607

[5] E. S. Alifia and O. R. Aji, "Analysis of the presence of coliform and *Escherichia coli* in ice cubes from beverage snacks at Pasar Tengah, Bandar Lampung," *Quagga: Jurnal Pendidikan dan Biologi*, vol. 13, no. 1, p. 74, 2020. [Online]. Available: https://doi.org/10.25134/quagga.v13i1.3698

[6] J. Saimin, H. Hartati, Y. Purnamasari, S. A. Mulyawati, Tien, and P. Ayitrina, "Microbiological and biochemical contamination analysis of refilled drinking water in Abeli, Kendari, Southeast Sulawesi," *Indonesian Biomedical Journal*, vol. 12, no. 2, pp. 124–129, 2020. [Online]. Available: https://doi.org/10.18585/inabj.v12i2.871

[7] M. K. Dipta, Budiyono, and N. A. Y. Dewanti, "Literature review: Risk factors for the presence of *Escherichia coli* bacteria in ice cubes," *Jurnal Kesehatan Masyarakat*, vol. 9, no. 3, pp. 377–385, 2021. [Online]. Available: https://doi.org/10.14710/jkm.v9i3.29585

[8] J. I. N. Sombie, J. Kagira, and N. Maina, "Prevalence and antibiogram of *Escherichia coli* and *Staphylococcus* spp. isolated from cattle milk products sold in Juja Sub-County, Kenya," *Journal of Tropical Medicine*, vol. 2022, art. no. 5251197, 2022. [Online]. Available: https://doi.org/10.1155/2022/5251197

[9] H. Hampikyan, E. B. Bingol, O. Cetin, and H. Colak, "Microbiological quality of ice and ice machines used in food establishments," *Journal of Water and Health*, vol.

15, no. 3, pp. 410–417, 2017. [Online]. Available: https://doi.org/10.2166/wh.2017.159

[10] Ministry of Health of the Republic of Indonesia, *Regulation of the Minister of Health of the Republic of Indonesia No. 2 of 2023 concerning Environmental Health Implementing Regulations*. Jakarta, Indonesia: Ministry of Health of the Republic of Indonesia, 2023. [Online]. Available: https://peraturan.bpk.go.id/Details/245563/permenkes-no-2-tahun-2023

[11] World Health Organization, *Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First and Second Addenda*. Geneva, Switzerland: World Health Organization, 2022. [Online]. Available: https://www.who.int/publications/i/item/9789240045064

[12] H. Hidayah, I. L. P. Mursal, H. A. Susaningsih, and S. Amal, "Analysis of coliform bacterial contamination and identification of *Escherichia coli* in block ice in Karawang City," *Pharma Xplore: Jurnal Ilmiah Farmasi*, vol. 7, no. 1, pp. 54–68, 2022. [Online]. Available: https://doi.org/10.36805/farmasi.v7i1.2335

[13] S. M. Yanestria *et al.*, "Antibiotic resistance pattern of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from broiler farm environment in Pasuruan District, Indonesia," *Biodiversitas Journal of Biological Diversity*, vol. 23, no. 9, 2022. [Online]. Available: https://doi.org/10.13057/biodiv/d230911

[14] Y. R. Mustika, M. H. Effendi, Y. Puspitasari, H. Plumeriastuti, A. R. Khairullah, and K. N. Kinasih, "Identification of multidrug-resistant *Escherichia coli* in cattle in abattoirs," *Journal of Medicinal and Veterinary Science*, vol. 7, no. 1, pp. 19–32, 2024. [Online]. Available: https://doi.org/10.20473/jmv.vol7.iss1.2024.19-32

[15] M. Bonnet, J.-C. Lagier, D. Raoult, and S. Khelaifia, "Bacterial culture through selective and non-selective conditions: The evolution of culture media in clinical microbiology," *New Microbes and New Infections*, vol. 34, art. no. 100622, 2020. [Online]. Available: https://doi.org/10.1016/j.nmni.2019.100622

[16] M. S. Hossain, V. Balakrishnan, N. N. N. A. Rahman, M. Z. I. Sarker, and M. O. A. Kadir, "Treatment of clinical solid waste using a steam autoclave as a possible alternative technology to incineration," *International Journal of Environmental Research and Public Health*, vol. 9, no. 3, pp. 855–867, 2012. [Online]. Available: https://doi.org/10.3390/ijerph9030855

[17] P. T. Hang, N. T. V. Quynh, N. K. Anh, and L. V. Thanh, "Effectiveness evaluation of autoclaves at the National Institute for Control of Vaccines and Biologicals," *Journal of Control Vaccines and Biologicals*, vol. 5, no. 3, 2025. [Online]. Available: https://doi.org/10.56086/jcvb.v5i3.227

[18] Y. Jiwintarum, A. Agrijanti, and B. L. Septiana, "Coliform most probable number (MPN) with varieties of media volume lactose broth single strength and lactose broth double strength," *Jurnal Kesehatan Prima*, vol. 11, no. 1, p. 11, 2018. [Online]. Available: https://doi.org/10.32807/jkp.v11i1.17

[19] I. P. M. A. P. P., "Microbiological examination of drinking water quality in the Bukit Jimbaran area," *CERATA: Jurnal Ilmu Farmasi*, vol. 12, no. 2, pp. 1–7, 2021. [Online]. Available: https://doi.org/10.61902/cerata.v12i2.200

[20] S. Shafi, A. N. Kamili, M. A. Shah, and S. A. Bandh, "Coliform bacterial estimation: A tool for assessing water quality of Manasbal Lake of Kashmir, Himalaya," *African Journal of Microbiology Research*, vol. 7, no. 31, pp. 3996–4000, 2013. [Online]. Available: https://doi.org/10.5897/AJMR2013.5863

[21] M. T. I. Farizqi *et al.*, "Detection of extended-spectrum β-lactamase-producing *Escherichia coli* genes isolated from cat rectal swabs at Surabaya Veterinary

Hospital, Indonesia," *Veterinary World*, pp. 1917–1925, 2023. [Online]. Available: https://doi.org/10.14202/vetworld.2023.1917-1925

[22] H. D. F. de Souza *et al.*, "Is green metallic sheen in EMB agar accurate to assist the *Escherichia coli* identification?," *Revista Pró-UniverSUS*, vol. 15, no. 4, pp. 1–9, 2025. [Online]. Available: https://doi.org/10.21727/rpu.v15i4.4520

[23] A. Widodo *et al.*, "Antibiotic sensitivity profile of multidrug-resistant *Escherichia coli* isolated from dairy cow's milk in Probolinggo, Indonesia," *Biodiversitas Journal of Biological Diversity*, vol. 23, no. 10, 2022. [Online]. Available: https://doi.org/10.13057/biodiv/d231002

[24] T. Ahmed, S. Baidya, M. Acharjee, and T. Rahman, "Qualitative analysis of drinking water through the most probable number (MPN) method," *Stamford Journal of Microbiology*, vol. 3, no. 1, pp. 9–16, 2015. [Online]. Available: https://doi.org/10.3329/sjm.v3i1.22745

[25] A. G. Bambang, Novel, and S. Kojong, "Analysis of coliform bacterial contamination and identification of *Escherichia coli* in refilled drinking water from depots in Manado City," *PHARMACON: Jurnal Ilmiah Farmasi UNSRAT*, vol. 3, no. 3, pp. 2302–2493, 2014. [Online]. Available: https://ejournal.unsrat.ac.id/index.php/pharmacon/article/view/5450

[26] F. Yevani, M. Y. Moi, and D. Ernaningsih, "Antibacterial strength of ethyl acetate extract of kligong leaves (*Crassocephalum crepidioides*) against the growth of *Escherichia coli* and *Staphylococcus aureus*," *Jurnal Syntax Admiration*, vol. 4, no. 1, pp. 1–16, 2023. [Online]. Available: https://jurnalsyntaxadmiration.com/index.php/jurnal/article/view/514

[27] I. Sayuti and Suratni, "Isolation and identification of hydrocarbonoclastic bacteria from petroleum liquid waste of GS Chevron Pacific Indonesia in Benar Village, Rimba Melintang District, Rokan Hilir," in *Proceedings of SEMIRATA*, pp. 320–334, 2015. [Online]. Available: https://jurnal.untan.ac.id/index.php/semirata2015/article/view/13752

[28] E. Hestianah *et al.*, "Rift Valley fever: A zoonotic disease with global potential," *Open Veterinary Journal*, vol. 15, no. 6, p. 2312, 2025. [Online]. Available: https://doi.org/10.5455/OVJ.2025.v15.i6.5

[29] B. Hadi, E. Bahar, and R. Semiarti, "Bacteriological test of household ice cubes used by beverage sellers in Lubuk Buaya Market, Padang City," *Jurnal Kesehatan Andalas*, vol. 3, no. 2, pp. 119–122, 2014. [Online]. Available: https://doi.org/10.25077/jka.v3i2.44

[30] T. Cahya, M. Amir, and R. R. Manalu, "Microbial contamination test of ice cubes from beverage sellers in the Jagakarsa Market area, South Jakarta," *Sainstech Farma*, vol. 12, no. 2, pp. 78–84, 2019. [Online]. Available: https://ejournal.istn.ac.id/index.php/saintechfarma/article/view/474

[31] F. Magfirah, E. Y. Mahtuti, and M. Masyhur, "Analysis of coliform bacteria in refilled drinking water depots in Jeru Village, Turen District," *Jurnal Kesehatan Masyarakat Celebes*, vol. 3, no. 3, pp. 1–7, 2022. [Online]. Available: https://journal.lppm-unasman.ac.id/index.php/jikm/article/view/2812