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POTENTIAL OF A MODIFIED CARY-BLAIR TRANSPORT MEDIUM USING KURISI FISH (Nemipterus sp.) FOR STOOL SAMPLES FROM DIARRHEA PATIENTS

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ABSTRACT

Diarrhea is a gastrointestinal infectious disease with a high prevalence, particularly among children. Accurate diagnosis requires transporting clinical samples to the laboratory using Cary-Blair transport medium. However, this medium is generally imported and relatively expensive. This study aimed to evaluate the potential of Kurisi fish waste (Nemipterus sp.) as a source of peptone in modified Cary-Blair transport medium as a more economical alternative. The study employed a laboratory experimental design using peptone produced through enzymatic hydrolysis of Kurisi fish waste with papain. The modified Cary-Blair medium was used to store 11 clinical stool samples from pediatric patients with diarrhea and was compared with a control Cary-Blair medium. The stored samples were cultured on selective media and examined macroscopically, microscopically, and biochemically. The bacteria identified in the modified medium were Salmonella sp. (46%), Shigella sp. (18%), and Escherichia coli (36%). These findings indicate that peptone derived from Kurisi fish waste has the potential to replace sodium thioglycolate in the Cary-Blair medium, offering a more affordable transport medium alternative while supporting the utilization of fish waste.
Keywords: Diarrhea; Cary-Blair; Peptone; Kurisi Fish Waste (Nemipterus sp.)

INTRODUCTION

Diarrhea is a gastrointestinal infectious disease that can affect individuals of all age groups; however, children are particularly vulnerable because their immune systems are not yet fully developed (Purnama, 2019). According to World Health Organization data in 2024, diarrhea affected 1.7 billion people and caused 443,832 deaths among children under five years of age. A 2023 report from the Banten Provincial Health Office indicated that Tangerang Regency had the highest number of diarrhea cases among children under five, with 42,290 cases (Dinas Kesehatan, 2023).

The high incidence of diarrhea highlights the importance of laboratory examination for accurate diagnosis and effective treatment. Stool cultures from patients with diarrhea are performed using Cary-Blair transport medium, which helps maintain sample quality during transport. However, this medium is generally imported from China, India, and the United Kingdom, at a price ranging from IDR 2–6 million per 500 grams. To address this issue, researchers have sought an alternative nitrogen source, namely peptone. Peptone is a mixture of polypeptides, dipeptides, and amino acids produced through the hydrolysis of protein-rich materials (Pratomo et al., 2020). Peptone derived from hydrolyzed fish protein has potential as a nitrogen source (Sunarno, 2020). Enzymatic peptone production uses proteolytic enzymes, such as papain from papaya latex, which effectively hydrolyze fish waste (Khoirunisa, 2023). According to Ghaly (2013), all parts of fish can be processed into peptone because their protein content can reach 57.92%. In addition, fish-processing industries generate 20%–80% waste, including heads, viscera, blood, and internal organs, which can be utilized as raw materials for peptone in modified media (Pratomo et al., 2020).

Several previous studies have utilized fish waste as a source of peptone. Pratomo et al. (2020) investigated peptone derived from Kurisi fish (Nemipterus sp.) waste and reported the growth of E. coli and S. aureus. Nurhayati et al. (2023) demonstrated that peptone derived from eel (Anguilla bicolor) viscera waste could support the growth of E. coli and S. aureus. In addition, Khoirunisa (2023) showed that the Cary-Blair transport medium could be modified with Kurisi fish (Nemipterus sp.) waste and was effective in supporting the growth of Salmonella typhi. These studies indicate that fish waste has potential as a substitute source of peptone. Therefore, this study investigated the use of peptone derived from Kurisi fish (Nemipterus sp.) waste in the Cary-Blair transport medium for clinical stool samples from patients with diarrhea.

MATERIALS AND METHODS

This study used a laboratory experimental design conducted at Poltekkes Kemenkes Banten, with sample collection at Tangerang Regency Hospital from March to May 2025. The peptone used in this study was prepared by hydrolyzing Kurisi fish waste with papain enzyme and was subsequently used as one of the components of the modified Cary-Blair medium. A total of 22 stool samples from children with diarrhea were collected; 11 samples were stored in the control medium and the other 11 samples were stored in the modified medium. The samples were then inoculated onto Blood Agar Plate, MacConkey Agar, and Salmonella Shigella Agar, followed by macroscopic and microscopic examination of bacterial growth and biochemical testing to identify the bacterial species.

RESULTS AND DISCUSSION

Results

Kurisi Fish Peptone (Nemipterus sp.)

The study yielded 70 mL of peptone from Kurisi fish waste, which was sufficient for preparing the modified Cary-Blair transport medium.



Figure 1 Peptone from Kurisi Fish Waste (Nemipterus sp.)

Preparation of the Modified Cary-Blair Transport Medium

The peptone derived from Kurisi fish (Nemipterus sp.) waste was subsequently used as a component of the modified Cary-Blair transport medium. To evaluate the performance of the modified medium containing Kurisi fish waste, a control medium was used for comparison.

Table 1 Comparison of Cary-Blair Transport Media

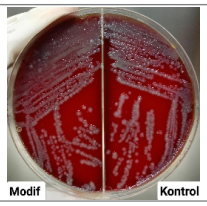
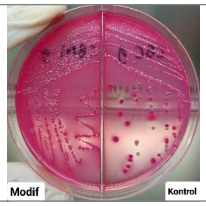
	Cary-Blair Control	**Modified Cary-Blair**
Cary-Blair Transport Medium		
Description	The medium was clear white, semi-solid in consistency, but	The medium was slightly cloudy white because it contained

	denser than the modified medium.	peptone, with a semi-solid consistency.

Macroscopic and Microscopic Observations

Samples inoculated onto BAP, MCA, and SSA media were subsequently examined macroscopically.

Table 2. Macroscopic Observations

	BAP Medium	**MCA Medium**	**SSA Medium**
Media Image			
Description	Round, white, convex colonies with smooth margins.	Round, pink, convex colonies with smooth margins.	Round, black, convex colonies with smooth margins.

Following macroscopic examination of the samples, cumulative bacterial growth data were compiled to compare the effectiveness of the modified medium with that of the control.

Table 3 Cumulative Bacterial Growth Data

Sample Code	**Modified Medium**	**Control Medium**	**Agreement Between Control and Modified Media**
Sample-01	Few black colonies	Many black colonies	18% Not consistent
Sample-02	Few black colonies	Many black colonies	18% Not consistent
Sample-03	White colonies	White colonies	82% Consistent
Sample-04	White colonies	White colonies	82% Consistent
Sample-05	White colonies	White colonies	82% Consistent
Sample-06	White colonies	White colonies	82% Consistent
Sample-07	White colonies	White colonies	82% Consistent
Sample-08	White colonies	White colonies	82% Consistent
Sample-09	Black colonies	Black colonies	82% Consistent
Sample-10	Black colonies	Black colonies	82% Consistent
Sample-11	Black colonies	Black colonies	82% Consistent

Based on the observations, 82% of the samples stored in the modified medium showed colony morphology and color similar to those observed in the control medium, whereas the remaining 18% showed discrepancies, as indicated by differences in colony numbers and medium color.

Microscopic examination was subsequently performed on all samples after Gram staining. The results showed that all samples (100%) contained Gram-negative rod-shaped bacteria.

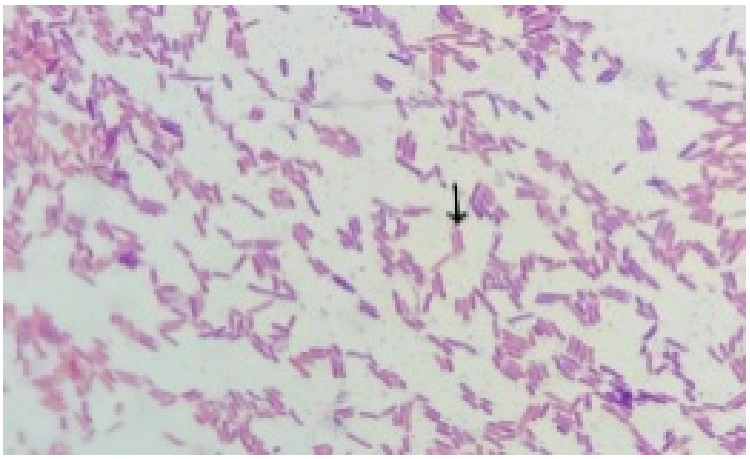


Figure 2 Microscopic Observation of Bacteria

Bacterial Identification by Biochemical Testing

To obtain bacterial data from the stool samples of diarrhea patients, biochemical tests were performed on colonies that grew on selective Salmonella Shigella Agar after being stored in the modified Cary-Blair medium. The identified isolates were coded CBM01 to CBM11 and are presented in the following table:

Table 4 Biochemical Testing

No.	Sample Code	Result	Bacterium
1	CBM01	Growth	Salmonella sp.
2	CBM02	Growth	Salmonella sp.
3	CBM03	Growth	Shigella sp.
4	CBM04	Growth	E. coli
5	CBM05	Growth	E. coli
6	CBM06	Growth	Shigella sp.
7	CBM07	Growth	E. coli
8	CBM08	Growth	E. coli
9	CBM09	Growth	Salmonella sp.
10	CBM10	Growth	Salmonella sp.
11	CBM11	Growth	Salmonella sp.

From the 11 samples tested, Salmonella sp. was detected in 5 samples, namely CBM01, CBM02, CBM09, CBM10, and CBM11, representing 46% of the total samples. Shigella sp. was detected in 2 samples, namely CBM03 and CBM06, representing 18% of the total. Escherichia coli was detected in the remaining 4 samples, namely CBM04, CBM05, CBM07, and CBM08, representing 36%.

Discussion

The study began with the preparation of peptone from Kurisi fish (Nemipterus sp.) waste, which serves as a primary nitrogen source for bacterial growth (Pratomo et al., 2020). The peptone was prepared through hydrolysis of Kurisi fish protein using papain as a catalyst. Proteolytic enzymes such as papain, bromelain, and alcalase can be used for protein hydrolysis. Papain is obtained from the latex of Carica papaya L. and specifically cleaves peptide bonds at the carbonyl group of amino acids within protein structures without damaging the amino acid structure (Ridhwan & Herdyastuti, 2024). The papain enzyme concentration used was 3.4%, referring to the study by Novitasari & Andriani (2021), which reported an optimum papain concentration of 3%–5%. This was further supported by Khoirunisa (2023), who reported that the use of 3.4% papain could produce optimal protein levels, particularly for peptone derived from Kurisi fish waste.

In the next stage, peptone derived from Kurisi fish waste was utilized as a component of the modified Cary-Blair transport medium, replacing sodium thioglycolate. According to Khoirunisa (2023), this peptone has potential as an alternative because it can inhibit oxidation processes. This finding is supported by Supriatin & Rahayyu (2020), who stated that peptone as a nitrogen source in modified Cary-Blair medium can maintain bacterial viability even in the absence of sodium thioglycolate. In addition to peptone, the modified Cary-Blair medium contains other components, including agar as a solidifying agent; sodium chloride and 1% calcium chloride to maintain osmotic pressure and prevent cell damage; and disodium hydrogen phosphate to maintain

a stable medium pH during sample storage and transportation (Khoirunisa, 2023). A control Cary-Blair medium was prepared for comparison. In laboratory experiments evaluating alternative media, the use of a commercial control is necessary because a good alternative medium should ideally approximate the function and performance of the control medium (Yuliana & Qurrohman, 2022).

After preparation of the modified and control Cary-Blair transport media, stool samples were collected from diarrhea patients at Tangerang Regency Hospital after informed consent had been obtained. No medical procedure was performed without the consent of the patients or their families (Rizka et al., 2023). The stool swab samples were immediately placed into the modified and control media and stored for 1–3 hours to simulate a delay in examination. According to Collier & Pence (2025), Cary-Blair medium can maintain bacterial viability for up to 48 hours.

The stool samples were then inoculated onto bacterial agar media for macroscopic observation. Bacterial growth on SSA was examined to assess the agreement between the control and modified media. Of the 22 samples tested, 82% showed colony morphology and color similar to those in the control medium, whereas 18% showed differences, including fewer colonies and changes in medium color in the modified medium compared with the control.

Nevertheless, differences in bacterial types were observed among the samples. Similar colony growth patterns were observed in Samples 01, 02, 09, 10, and 11, characterized by a change in the medium color to yellow with black colonies, indicating the presence of Salmonella sp. through H_2S production (Nugroho et al., 2025). Meanwhile, Samples 03, 04, 05, 06, 07, and 08 showed a yellow color on SSA without H_2S production, but with white colonies. According to Alifya et al. (2022), white or translucent colonies on SSA indicate non-lactose-fermenting bacteria that lack thiosulfate reductase activity and do not produce H_2S gas. Therefore, microscopic and biochemical examinations were necessary to identify the bacterial species present.

Microscopic examination was performed by Gram staining of colonies obtained from SSA. The microscopic observations showed that all samples (100%) contained Gram-negative bacilli, characterized by a pink color of the cell walls. According to Istiqomah et al. (2023), Salmonella, Shigella, and E. coli are known to be Gram-negative rod-shaped bacteria. These bacteria have a thin peptidoglycan layer and an outer cell membrane composed of lipopolysaccharides (Khoirunisa, 2023).

Biochemical testing was subsequently performed on 11 samples from SSA. Salmonella sp. was detected in 5 samples (46%), namely CBM01, CBM02, CBM09, CBM10, and CBM11. Shigella was detected in 2 samples (18%), namely CBM03 and CBM06. In addition, Escherichia coli was detected in 4 other samples (36%), namely CBM04, CBM05, CBM07, and CBM08, on SSA. Although SSA is selective for the isolation of Salmonella and Shigella, the medium can still allow the growth of other bacteria such as E. coli (Alifya et al., 2022), particularly because stool samples may contain more than one type of bacterium.

Based on the identification results, one limitation of this study was that bacterial growth in the modified medium tended to be less optimal, as indicated by the 18% of samples in the modified medium that showed fewer colonies than the control medium. This finding is consistent with Khoirunisa (2023), who reported that bacteria were able to survive in the modified medium, although the number of colonies was lower than in the control medium. Nevertheless, overall, the modified medium was still able to maintain bacterial viability in most samples.

CONCLUSION

Peptone derived from Kurisi fish (Nemipterus sp.) waste showed potential as an alternative ingredient for the preparation of modified Cary-Blair medium. This was supported by the identification of microorganisms growing in the medium, including Salmonella sp. (46%), Shigella sp. (18%), and Escherichia coli (E. coli) (36%). Macroscopically, 82% of the colonies showed similar morphology, namely a round shape, convex surface, and smooth margins. This morphological similarity indicates that the medium used had a stable capacity to support bacterial growth. The remaining 18% showed differences in the number of colonies that grew. Microscopically, all samples (100%) exhibited Gram-negative, rod-shaped bacterial characteristics, consistent with the general characteristics of the three identified bacterial species.

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