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Density of *Escherichia coli* Bacteria in Blood Clams (*Anadara granosa*) in Anak Setatah Village, Meranti Islands Regency, Riau

Densitas Bakteri Escherichia coli pada Kerang Darah (Anadara granosa) di Desa Anak Setatah, Kabupaten Kepulauan Meranti, Provinsi Riau

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Abstract

Blood clams are soft-bodied animals that live sessile and obtain food by filtering (filter feeders). This results in many microorganisms accumulating in the body of the blood clams, one of which is the coliform bacteria group. *Coliform* bacteria are divided into two types, namely fecal coliform and non-fecal coliform. Fecal coliform comes from animal and human feces, such as *Escherichia coli*. According to SNI 01-7388-2009, the MPN value of *E. coli* bacteria for fresh *Mollusca*, *Crustaceans*, and *Echinodermata* is <3/g. This study aimed to calculate the density of *E. coli* bacteria in blood clams. This study was conducted from December 2024 to February 2025. Sampling was conducted at the lowest ebb with one transect consisting of 3 plots measuring 1 × 1m². The MPN method is a method for analyzing coliform bacteria and *E. coli*. The results of the estimation test obtained at station 1, namely the Tebing Dian Beach Area, ranged from 7-10 MPN/g with an average of 12.6 MPN/g and station 2, namely the "Cinta Mangrove" Tourism Area, ranged from 4-15 MPN/g with an average of 10 MPN/g. While the results of the estimation test obtained at station 1 ranged from 3-4 MPN/g with an average of 2.3 MPN/g, stations 2 ranged from 3 MPN/g with an average of 1.0 MPN/g. These results indicate that the abundance of *E. coli* bacteria in blood clams does not exceed the quality standards set based on SNI 01-7388-2009.

Keywords: Density, Blood Clams, Coliform, *E. coli*, MPN Method.

Abstrak

Kerang darah merupakan salah satu jenis hewan bertubuh lunak yang hidup menetap (*sessile*) dan memperoleh makanan dengan cara menyaring (*filter feeder*). Hal ini mengakibatkan banyaknya mikroorganisme yang terkumpul di dalam tubuh kerang darah, salah satunya yaitu kelompok bakteri *Coliform*. Bakteri *Coliform* dibagi menjadi dua yaitu fecal coliform dan non fecal coliform. Fecal Coliform berasal dari kotoran hewan dan manusia, seperti bakteri *Escherichia coli*. Menurut SNI 01-7388-2009 nilai MPN bakteri *E. coli* untuk *Mollusca, Crustasea*, dan *Echinodermata* segar adalah <3/g. Tujuan dari penelitian ini adalah untuk menghitung densitas bakteri *E. coli* pada kerang darah. Penelitian ini dilaksanakan pada bulan Desember 2024 sampai Februari 2025. Pengambilan sampel dilakukan saat surut terendah dengan 1 transek yang terdiri dari 3 plot berukuran 1 × 1m². Metode MPN merupakan metode untuk menganalisi bakteri *coliform* dan *E. coli*. Hasil uji pendugaan yang didapat pada stasiun 1 yaitu Kawasan Pantai Tebing Dian yang berkisaran 7-10 MPN/g

dengan rata-rata 12,6 MPN/g dan stasiun 2 yaitu Kawasan Wisata "Cinta Mangrove" yang berkisaran 4-15 MPN/g dengan rata-rata 10 MPN/g. Sedangkan hasil uji pendugaan yang didapat pada stasiun 1 yang berkisaran 3-4 MPN/g dengan rata-rata 2,3 MPN/g sedangkan 3 stasiun 2 berkisaran 3 MPN/g dengan rata-rata 1,0 MPN/g. Hasil tersebut menunjukkan bahwa kelimpahan bakteri *E. coli* pada kerang darah tidak melebihi baku mutu yang ditetapkan berdasarkan SNI 01-7388-2009

Kata kunci: Densitas, Kerang Darah, Coliform, *E. coli*, Metode MPN.

1. Introduction

Blood clams are one type of shellfish with economic value and can be used as a source of protein and minerals to meet the community's food needs. To survive, blood clams interact with the environment and choose the best environmental conditions and habitat types to continue growing and reproducing. Blood clams live in muddy waters and eat plankton and benthic organisms (Simanjuntak, 2020). In addition, this shellfish is also called a filter feeder because it obtains food by eating particles from organic matter suspended in water by passing water into the body (Emawati et al., 2015). Suspension-feeding bivalves such as blood clams can reduce water turbidity through filtering activities, fertilize benthic habitats through bio-deposition, induce denitrification, overcome some of the detrimental effects of eutrophication in shallow waters, absorb carbon, provide structural habitat for other marine organisms, and stabilize habitats and coastlines (Santoso, 2022).

Escherichia coli bacteria are one type of coliform bacteria found in the digestive organs of humans and animals. *E. coli* bacteria in food and drinks are highly correlated with the discovery of disease germs (pathogens). The presence of *E. coli* bacteria in animals or humans increases; these bacteria will produce enterotoxins that can cause diarrhea, urinary tract infections, and gastrointestinal infections (Najah & Bintari, 2021). *Escherichia coli* can survive at high acidity levels in the human body. These bacteria can also survive outside the human body, spreading through feces. These two habitats of *E. coli* are quite opposite (Arif, 2022). The human digestive tract is a relatively stable, warm, anaerobic, and nutrient-rich habitat. Meanwhile, outside the digestive tract, environmental conditions can vary greatly; they can be much colder, aerobic, and contain fewer nutrients (Rahayu et al., 2021). The presence and spread of *E. coli* bacteria in blood clams can be affected by water conditions, such as current speed, temperature, brightness and salinity, so it is necessary to measure water quality.

Meranti Islands Regency, especially Anak Setatah Village, is a coastal area rich in marine resources, including blood clams (*Anadara granosa*), which are widely used by the community. However, the potential for bacterial contamination, especially Escherichia coli, is a concern because it can endanger consumer health. Many studies have been conducted on *E. coli* bacteria in seashells. However, there has been no research on *E. coli* bacteria in blood clams in Anak Setatah Village. The main objective of this study was to calculate the density of *E. coli* bacteria in blood clams.

2. Materials and Methods

2.1. Time and Place

This research was conducted in December 2024- February 2025. Blood clam samples were obtained from the coast of Anak Setatah Village, Meranti Islands Regency, Riau Province (Figure 1). Furthermore, it was studied at the Marine Microbiology Laboratory, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Universitas Riau.

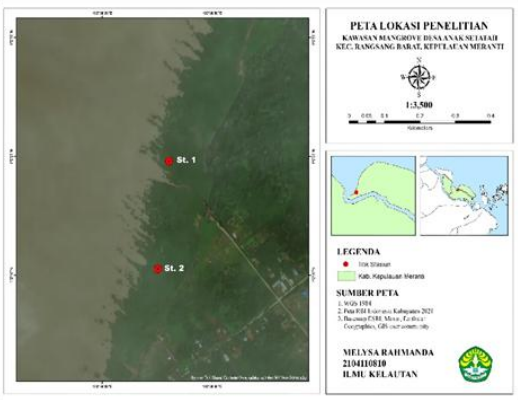

Figure 1. Research location

2.2. Methods

The research location was determined using the purposive sampling method. Sampling was carried out at two stations, namely station 1 located around Tebing Dian Beach, Anak Setatah Village and station 2 located around the "Cinta Mangrove" Tourism Area, Anak Setatah Village. Each research station has one transect consisting of 3 plots measuring 1 × 1m², with a distance between plots of 10 m. The determination of the research location can be seen in Figure 2.

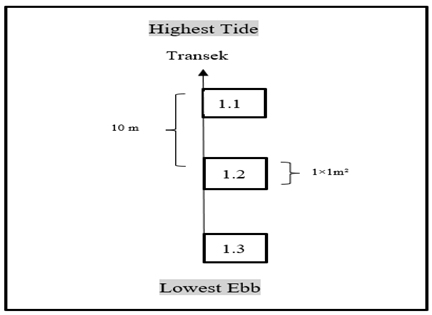

Figure 2. Research plot scheme

2.3. Procedures

2.3.1. Sampling

Blood clam sampling was conducted at the lowest ebb with 10 samples in each plot. Sampling of clams using a stick measuring ± 2 m, clam samples were taken randomly by hand, after which the samples were put into an open container that had been labelled with information on the location of the station and plot. Then taken to the laboratory for analysis.

2.3.2. Measurement of Water Quality Parameters

Parameters are measured using appropriate instruments. Temperature is measured using a thermometer, salinity is measured using a refractometer, while the degree of acidity (pH) is measured using pH indicator paper. Measurement of these parameters is vital to understanding the environmental conditions affecting blood clams' growth and survival.

2.3.3. Research Procedures in the Laboratory

Sample analysis was carried out with the following procedures: 1) The glass tool was washed first and dried then wrapped using rice paper, then sterilized using an autoclave at a temperature of 121°c with a pressure of 2 Atm for 15 minutes; 2) Clean the shells using running water; 3) The meat was taken as much as 10 grams and mashed; 4) Dilution was carried out from 10-1 to 10-3; 5) Isolation of Escherichia coli bacteria was carried out by three tests including a presumptive test using lactose broth media, a confirmation test using brilliant green lactose broth media and a complementary test using eosin methylene blue agar media.

2.3.4. Bacterial Analysis

Bacterial analysis was conducted using the Most Probable Number (MPN) method of a 3-tube series based on the Indonesian National Standardization Agency (SNI) No. 2897; 2006. The statement of the results of the MPN test, namely the number of tubes that were positive for gas, was recorded and referred to the MPN table of the 3-tube series. The numbers obtained in the MPN table indicate the number of coliform bacteria in each gram/ml of the sample tested (Silaban, 2019).

CFU/mL = value of MPN x 1/(Middle tube dilution)

2.3.5. Observation of Bacterial Morphology

Observation of bacterial colony morphology by paying attention to the color, shape of the colony, edges and elevation of the bacteria.

2.3.6. Biochemical Activity Test

Bacteria have various biochemical activities by using nutrients obtained from their environment. Biochemical transformations can occur inside and outside the bacteria, regulated by enzymes. Each bacterium can use its enzymes to degrade carbohydrates, fats, proteins, and amino acids. Some biochemical activity tests that are carried out are as follows: 1) Catalase test; 2) Sugar staining; 3) Sulfide test (H_2S); 4) Sugar utilization test; 5) Motility test; 6) Indole test; 7) citrate test; 8) methyl red test (MR).

2.4. Data Analysis

The data obtained are presented in tables, and then the data is discussed descriptively. The data obtained is supported by references related to this study.

3. Result and Discussion

3.1. Water Quality Parameters

The research location's water quality was measured after sampling and at the lowest ebb. The water quality parameters measured during the study were temperature ranging from 28 to 30℃, pH ranging from 6 to 7, and salinity ranging from 23 to 24 ppt. In measuring water quality parameters in the waters of the Anak Setatah village, there was a slight difference at the two stations due to several factors such as water conditions at the time of measurement, activities near the waters that can affect seawater quality and the time of measurement. Water quality parameters in the waters of Anak Setatah Village can be seen in Table 1.

Table 1. Water quality parameters in the waters of Anak Setatah Village

Parameters	Station 1	Station 2
Suhu (℃)	35	28
pH	7	6
Salinity (ppt)	24	23

Temperature is one of the critical factors in water that affects the metabolism of aquatic organisms. Based on PP No. 22 of 2021, the appropriate seawater temperature for marine life ranges from 28-31℃. At the same time, the optimum temperature for the growth of *E. coli* bacteria is 37℃, but can also grow in the range of 15-45℃. Temperature significantly affects the rate of microbial growth and enzyme activity. Every microbe, including bacteria, has an optimum, maximum and minimum temperature for its growth. If the environmental temperature exceeds the minimum and maximum temperature thresholds for growth, enzyme activity will stop; even at temperatures too high, enzyme denaturation will occur (Arvino & Nurul, 2017).

The acidity (pH) values of the two sampling stations follow PP No. 22 of 2021, which states that for the life of aquatic organisms, the recommended pH value of waters is 6-9. The results of this study indicate that the pH of the waters obtained in the observation area is classified as unpolluted and has not been disturbed by its surroundings. The acidity level that bacteria need to grow well is mainly at a neutral pH.

Salinity affects the osmotic pressure on the bacterial cell wall. High salinity levels can damage the cell wall and cause death to bacteria. The salinity value still supports the life and growth of *E. coli. E. coli* bacteria cannot survive long and can even cause death at relatively high salinity >30 ppt (Afianti & Sutiknowati, 2020). *E. coli* can grow optimally without salt content, but at low salt levels (Genisa & Auliandri, 2018).

3.2. Density of E. coli Bacteria

Escherichia coli isolation was carried out using the MPN method with three stages of testing, namely the presumptive test, the confirmatory test and the complementary test. The results of the presumptive test and the confirmatory test refer to the MPN table of 3 series of tubes to determine the number of bacteria contained, as seen in Tables 2 and 3**.**

Table 2. Presumptive test

Station	Sample code	Series	Index MPN/g
1	P.1	2-1-1	20
	P.2	1-2-0	11
	P.3	1-0-1	7
Average			12,6
2	P.1	2-1-0	15
	P.2	1-2-0	11
	P.3	1-0-0	4
Average			10

Table 3. Confirmed test

Station	Sample code	Series	Index MPN/g
1	P.1	1-0-0	4
	P.2	0-0-0	0
	P.3	0-0-1	3
Average			2,3
2	P.1	0-0-0	0
	P.2	0-1-0	3
	P.3	0-0-0	0
Average			1,0

Coliform ferments lactose, which causes turbidity and gas bubbles in the tube. The change in media becomes cloudy due to increased acidity, resulting in the coagulation of lactose components. Meanwhile, the presence of gas bubbles is due to lactose fermentation, which produces CO_2 gas (Alang, 2014). The presence of coliform bacteria indicates that the environmental conditions in the waters have also decreased biologically. Coliform density that exceeds the seawater quality standard threshold can indicate high pollution and become pathogenic to the existence of biota in the waters (Annisa et al., 2024).

Result of the estimation test as presented in Table 2, shows that the highest abundance of *coliform* bacteria was found in Station 1 (Tebing Dian Beach area), which ranged from 7-20 MPN/g with an average of 12.6 MPN/g, while the lowest number of coliforms was at Station 2 ("Cinta Mangrove" tourist area), which ranged from 4-15 MPN/g with an average of 10 MPN/g. Mussels are filter feeders, that is, they obtain their food by filtering plankton, microorganisms and organic matter particles found in the water, thus allowing for contamination of heavy metals dissolved in water and accumulating in the blood of the mussels (Katon et al., 2020). Generally, the number of E. coli bacteria found in mussels is quite high because they have the property of accumulating particles found in water.

The highest abundance of coliform bacteria was found in the Dian cliff beach with an average of 2.3 MPN/g, compared to that in the "Cinta Mangrove" tourist area with an average of 1.0 MPN/g. Saleem et al. (2024) explained that the source of coastal contamination is greater in receiving runoff from land containing domestic waste, the primary source of fecal coliform bacteria. Mangrove areas tend to be more protected from direct sources of pollution because vegetation functions as a natural filter. The results obtained in the complementary test for this blood clams sample indicated that *E. coli* bacteria were present at all stations, with three isolates. This was obtained from scratching positive confirmation test samples on EMBA media, as indicated by a metallic green color on the scratching results. The results of bacterial inoculation from BGLBB media to EMBA media showed a color change by producing metallic green bacterial colonies, indicating the presence of *E. coli* (Susilo et al., 2022).

3.3. Morphological and Physiological Characteristics of Bacteria

The morphological characteristics of E. coli bacteria are identified macroscopically by observing the shape of the colony, elevation, edges, and color. Microscopic morphological observations are also carried out using Gram staining, which aims to determine bacteria's cell shape and Gram properties. Observations of bacterial morphology can be seen in Table 4.

Table 4. Morphological characteristics of bacterial isolates

Isolate code	Morphological characteristics					
	Colony Form	Elevation	Edge	Color	Cell Shape	Gram Properties
S.1 P.1	Irregular	Convex	Undulate	Metallic Green	Stem	Negative
S.1 P.3	Circular	Arise	Entire	Metallic Green	Stem	Negative
S.2 P.2	Circular	Arise	Entire	Metallic Green	Stem	Negative

Based on the results of macroscopic morphological observations in Table 4, it was found that the shape of the bacterial colonies in the three isolates observed was round (circular) and irregular. Then, the elevation obtained was raised and convexed. The edges of the bacteria obtained from the observation results were wavy (undulate) and smooth (entire), and the color of the bacterial colonies obtained was metallic green. Metallic green colonies indicate that the growing bacteria can ferment lactose and produce acid products generally characterized by a metallic sheen (Wicaksono, 2016).

The red color in bacterial cells indicates gram-negative bacteria. This is because Gram-negative bacteria have a cell wall composition containing more lipopolysaccharides than Gram-positive bacteria, so that these bacteria do not retain crystal violet. However, when stained with safranin, these bacteria will retain the safranin color as red (Ummamie et al., 2017). *E. coli* bacteria are rod-shaped, do not produce spores, are Gram-negative and have facultative anaerobic properties (Kumar et al., 2013). The physiological characteristics of bacteria were identified using biochemical tests consisting of catalase test, H_2S test, sugar utilization test, motility test, indole test, citrate test and methyl red test. The results of the biochemical tests conducted on three bacterial isolates can be seen in Table 5.

Table 5. Biochemical characteristics of *E. coli* isolates

Sample	Biochemical characteristics						
	Catalase	*Sulfide*	*Use of Sugar*	*Motility*	*Indole*	*Citrate*	*Methyl Red*
S.1 P.1	+	+	A/A	+	+	+	+
S.1 P.3	+	-	A/A	+	+	-	+
S.2 P.2	+	-	A/A	+	+	-	+

Sulfide Indole Motility (SIM) Media conducted three tests: sulfide, indole and motility. This test used three suspected *E. coli* isolates, namely metallic green colonies on EMBA media. Of the three isolates tested, one negative isolate produced sulfide, indicated by the presence of a black precipitate, indicating that the microorganism could produce H_2S and two negative isolates produced sulfide. The indole test is to see the ability of bacteria to convert the amino acid tryptophan using the tryptophanase enzyme, which produces indole (Wulan, 2019). A positive result is indicated by forming a red ring at the top of the media after being dripped with the Kovax reagent.

Of the three isolates inoculated on TSIA media, there were three isolates on the slant and butt sections that changed color to yellow A/A (Acid/Acid) with three isolates of media lifting or cracking. The color change in the media to yellow indicates that the bacteria can ferment all types of sugar. While the color of the media becomes yellow-red, indicating that the bacteria can ferment glucose-type sugar. And the lifted media indicates that the bacteria produce gases such as H_2 and CO_2 from the fermentation results. This is for media that contains black sediment because the media reacts with H_2S. Bacteria belonging to the genus *Escherichia* will make the media turn yellow on the slant by producing visible gas from the media as if it is broken or split and does not produce H_2S (Sari et al., 2019).

Of the three isolates, only one was positive for citrate, which was indicated by a change in the color of the culture medium from green to blue, while the two negative citrate isolates did not experience a color change. Positive citrate indicates that the bacteria can utilize citrate as a carbon and energy source. Meanwhile, *E. coli*

bacteria have properties that prevent them from using citrate in the media. Methyl Red (MR) test was conducted to determine the ability of bacteria to ferment glucose by producing acid after being dripped with methyl red reagent due to the decrease in the pH of the growth medium to 5.0 or lower (Sari et al., 2019). The results of the MR test on three isolates showed positive results in all isolates, which were indicated by a change in red color after being dipped in methyl red solution. *E. coli* bacteria have biochemical characteristics that are indole positive and motile, produce catalase enzymes, do not utilize citrate as a carbon source (citrate negative) and can produce acid (methyl red positive) (Putri, 2016).

4. Conclusions

Based on the results of this study, it can be concluded that the density of bacteria in blood clams at two sampling stations with the number of *Escherichia coli* bacterial cells does not exceed the quality standards or thresholds that have been established based on SNI 01-7388-2009, with the highest average at station 1 in the Tebing Dian coastal area of 2.3 MPN/g and the lowest average at station 2 in the "Cinta Mangrove" mangrove area of 1.0 MPN/g. Observations of water quality parameters at station 1, temperature 35 ℃, pH 7 and salinity 24 ppt, while at station 2, temperature 28 ℃, pH 6 and salinity 23 ppt. Based on PP No. 22 of 2021, the value of the seawater quality parameters above is still suitable for the life of marine biota and the development of bacteria.

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