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Identification of Escherichia Coli Bacteria in Well Water Treatment at STIKes Namira Madina and Bottle Drinking Water

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ABSTRACT

Well water is one of the main sources of clean water supply for residents in STIKes Namira Madina. Well water has bacteriological qualities that are easily contaminated. Well water provides water that comes from the soil layer relative to the ground surface. Based on the Republic of Indonesia Minuster of Health Regulation no. 492 of 2010 concerning drinking water quality requirements states that the content of Escherichia coli bacteria in drinking water is 0/100 ml and for well water 10/100 ml. Drinking water and well water must not exceed specified requirements. This research aims to determine the quality of well water at STIKes Namira Madina and bottled drinking water. This research used descriptive experimental methods, sampling, bacterial identification, Gram staining, biochemical reaction tests, sugar medium test, triple sugar iron agar medium test, sulfur indole moltility medium test, urea agar medium test. of the 4 samples tested, 1 sample met the drinking water quality requirements according to the PERMENKES and the other 3 samples contained Escherichia coli bacteria in the well water at STIKes Namira Madina. The result of the 4 samples tested was only 1 sample that was suitable for drinking while the other 3 samples were not 3 samples contained Escherichia coli bacteria.

Keywords: *Well water at STIKes Namira Madina, bottled drinking water, Escherichia coli bacteria.*

1. INTRODUCTION

Water is at natural resources with a very important function in human life. The uses of water in the human body includes helping the digestive process, regulating metabolism, transporting nutrients in the body, regulating body balance, and preventing the body from drying out. According to doctors and health experts, the human body needs 2.5 liters of water to be consumed or the equivalent of eight glasses every day (Asiska Permata Dewi ddk, 2021).

According to the Indonesian Minister of Health Regulation no. 492/Menkes/IV/2010, water has gone through a processing process or without a processing process that meets health requirements and can be drunk directly. The requirements for drinking water that are safe for health if they meets the physical, microbiological, chemical, and radioactive requirements are contained in the mandatory parameters and additional parameters (Reny Salim ddk, 2021).

Based on the Indonesian Minister of Health Regulation No. 492 of 2010 concerning drinking water quality requirements, the content of Escherichia coli in drinking water is 0/100 ml and 10/100 ml for well water. Drinking water and well water must not exceed the specified requirements.

Bottled drinking water is raw water that has undergone a sterilization process, is packaged, and is safe to drink, including mineral water and demineralized water. In recent years, the sale of bottled drinking water in Indonesia has grown very rapidly, so there is a lot of competition on how to produce drinking water suitable for public consumption (Vita Meylani ddk, 2019).

Bottled drinking water products must meet the mandatory quality requirements of the Indonesian National Standard (SNI) and obtain state permits from the Food and Drug Supervisory Agency (BPOM). There

is a mandatory reference for the quality standards of bottled drinking water approved by the BPOM, namely (SNI)-01-3553-2015, and the test parameters used as quality references are listed (Eka Bela Corisa ddk, 2022).

Escherichia coli ATCC 33456 is a gram-negative bacterium belonging to the Enterobacteriaceae family, which are found in the human body. They move using flagella and are short, rod-shaped, or commonly known as coccobacilli. Bottled drinking water can also be harmful if contaminated by Escherichia coli ATCC 33456 bacteria because people cannot drink bottled water that has been contaminated by bacteria because it can cause diarrhea (Muh Taufiqurrohmanddk, 2023).

Dug well water is one of the main sources of clean water for residents living in the rural and urban areas of Indonesia. Dug wells have bacteriological qualities that make them easily contaminated. Dug wells provide water from layers of soil that are relatively close to the surface of the ground.

Therefore, dug wells are easily contaminated through seepage from human and animal wastes; or domestic household needs (Siti Nurkumala Sari et al., 2019).

Sources of pollution adjusted to the direction of groundwater flow around STIKes, Namira Madina. The results showed that there were three sources of pollution in the well. The most dominant types of pollution sources around STIKes Namira Madina are close to septic tanks, rice fields, and drainage channels (Nurkumala Sari ddk, 2019).

Based on previous research conducted by Siti Nurkomala Sari ddk, entitled Identification of Escherichia coli Bacteria in Dug Well Water in Kelapa Tiga, Kaliawi Persada, and Pasir Gantung Villages, Bandar Lampung City in 2019. The results of this study showed that in 21 samples, positive results were obtained for all samples.

Based on the background and previous research, the researcher is interested in conducting a study entitled Identification of Escherichia coli ATCC 33456 Bacteria in Well Water Processing at STIkes Namira Madina and Packaged Drinks to determine the presence of E. coli ATCC 33456 bacteria and to determine the density of Escherichia coli ATCC 33456 bacterial cells in well water at STIkes Namira Madina and packaged water.

2. METHOD

This research was conducted in a qualitative experimental manner. The research included, sampling, bacterial identification, Gram staining, planting on selective media, biochemical reaction tests, (sugar-sugar medium test, simoncitrat medium test, triple sugar iron agar (TSIA) medium test, sulfur indolmoltility (SIM) medium test, and urea agar medium test). This research was conducted at the Microbiology Laboratory of STIKes, Namira Madina, Panyabungan. This research was conducted at the Microbiology Laboratory of STIKes Namira Madina Panyabungan in May 2024. The population and sample in this study were well water at STIKes Namira Madina in as many as three wells. The collected data analysis technique processed and presented in tabular form. Furthermore, the results were compared with the drinking water requirements based on the Regulation of the Minister of Health of the Republic of Indonesia Number 492 / Menkes / Per / IV / 2010.

Tool

Autoclave, incubator, sample bottle, loop needle, test tube rack, Durham tube, laminar air flow, pipette filler, micro-pipette, label paper, Bunsen lamp, gauze, microscope, 100 ML measuring cup, 100 ML glass beaker, thread, petri dish.

Material

Eosin Methylene Blue (EMB), alcohol, distilled water, drinking water samples, mannitol salt agar (MSA), brain heart infusion (BHI), sulfur indole motility (SIM), urea agar, triple sugar iron agar (TSIA), Simon citrate (SC), safranin, immersion oil, lugol, gention violet, and well water samples.

3. RESULTS AND DISCUSSION

Well Water Collection at STIkes Namira Madina

This research was conducted in a qualitative experimental manner. Well water samples from STIKes Namira Madina were collected on Jl. Bhayangkara No. 125 Panyabungan Natal, North Sumatra, Mandailing Natal Regency, and bottled drinking water around Panyabungan. Sampling was conducted in May 2024. These are found at three well points in STIKes Namira Madina, namely samples A, B, C, and D of bottled drinking water.

Results of Bacterial Identification in the Growth Phase of Escherichia ecoli in Samples

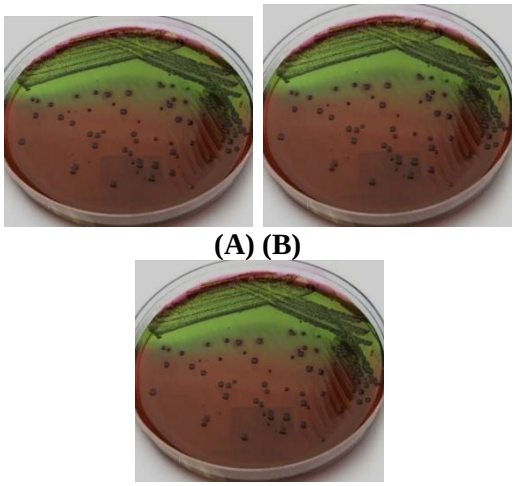
Because EMBA medium contains eosin, it can give a metallic green color to gram-positive bacteria, whereas Escherichia coli is a gram-negative bacterium that can reproduce very quickly.
Bacterial identification is the first step in a series of studies on the exploration and utilization of bacteria that grow in an area. Identification can be performed conventionally through biochemical and microscopic characterization of bacterial cells on a moleculer basis. Bacterial identification aims to identify bacterial colonies that are metallic green.
The working procedure for bacterial identification was to pipette 1 ml of sample into Braint Heart Infusion medium by dipping it into a tube containing Braint Heart Infusion medium, followed by incubation in an incubator at 370C for 24 h. If turbidity occurred, bacterial growth was indicated.

Table 1. The results of the identification of Escherichia coli bacteria in well water at STIkes Namira Madina using EMBA (Eosin Methylene Blue Agar) media.

Sample	Results	Results Based on Literature
A	+	Blackish blue/metallic green
B	+	Blackish blue/metallic green
C	+	Blackish blue/metallic green

Information:
(+) : Positive samples for Escherichia coli bacteria
(-) : Negative samples for Escherichia coli bacteria
Based on Table 1, the results of identifying the presence of Escherichia coli bacteria
In the well water sample at STIKes Namira Madina, it can be seen that the well water sample at STIKes Namira Madina tested positive for contamination with Escherichia coli bacteria in the well water sample. Therefor, it can be concluded that the well water sample at STIKes Namira Madina was contaminated with Escherichia coli bacteria because it was close to the septic tank, rice fields, and soil.

Figure 1. Results of identification of Escherichia coli bacteria in bottled water



(A) (B)

(C)

Figure 2. The results of the identification of Escherichia coli bacteria in well water A, B and well water D at STIKes Namira Madina (+).

From Figures 1 and 2, it can be seen that well water at STIkes Namira Madina and bottled water can be distinguished from the results of observations that can be seen in the Escherichia coli bacteria, where the difference in well water indicates bacterial growth, and in bottled water there is no sign of bacterial growth.

The content of lactose and eosin dyes and methylene blue can be used to distinguish between bacteria that can ferment lactose and non-fermenters. Positive results on EMBA medium will appear blackish blue with

metallic green, caused by the large quantity of acid produced and the deposition of dyes on the surface of bacterial growth.

Gram Staining on Samples

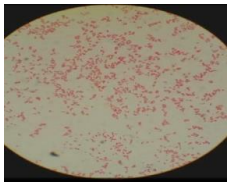
Gram staining of bacteria is a strategy to determine the morphology of microscopic organisms and, to identify and determine whether bacteria are gram-positive or gram-negative.

Gram staining aims to determine and identify the morphology of bacterial cells and determine whether the microscopic organism is gram-positive or gram-negative. Microscopic organisms of Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content, whereas gram-negative microorganisms have a cell wall structure with a high lipid content.

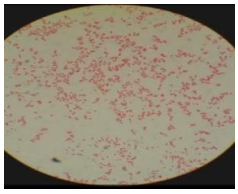
The working procedure of Gram staining is to sterilize the inoculating loop on the fire on the Bunsen flame until it turns red and, then wait for it to cool for approximately 30 s. Drop the gention violet dye liquid on the preparation evenly, wait for 1 min, rinse with running water, remove with 95% alcohol, drop again with safranin for 1 min, then wash with running water and dry, drop immersion oil, and examine under a microscope. If the bacteria are purple, it means gram-positive bacteria, and if the bacteria are red, it means gram-negative bacteria.

The results of Gram staining can be seen in the image below:

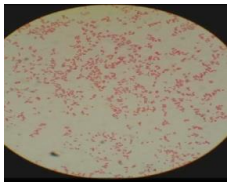
Figure 3. Results from bottled drinking water



(A)



(B)



(C)

Figure 4. The results of Gram staining of well water A, B and well water C at STIkes Namira Madina

From the picture, it can be seen that the type of bacteria is observed under a microscope using a 10x ocular lens, so that the total magnification is 100x magnification. Based on the results of Gram staining, Escherichia coli bacteria were shown to have red and rod-shaped morphology. Muh Tufiqurrahman ddk (2023) states that Escherichia coli bacteria are negative Garm bacteria in the form of short, straight rods, commonly called cocobasil.

When Gram staining is carried out, Escherichia coli bacteria cannot absorb the gention violet color, because they have a thin cell wall layer; thus, after adding alcohol, it will disappear and instead absorb the red color from safranin.

Biochemical Reaction Test of Sugar Medium

The sugar medium test can ferment sugar, especially carbohydrates. To determine the nature of bacterial fermentation of certain sugars. The sugar medium test aims to determine the nature of bacterial fermentation of certain sugars.

In the sugar medium test works is that 1 colony loop on EMBA medium was cultured on glucose, lactose, maltose, and sucrose media and incubated at 370C for 24 h. Observation was performed by looking for a change in color in the sugar medium, which was originally red to yellow. If the medium changed color from red to yellow, positive bacterial growth in the sugar medium was indicated.

Biochemical reaction tests were conducted to identify the Enterobacteriaceae bacterial family, in which Escherichia coli bacteria were included in the family group. The results of the sugar medium tests are shown in table 3.

Table 3. Sugar Media Test

Sample	Lactose	Glucose	Maltose
A	+	+	+
B	+	+	+
C	+	+	+

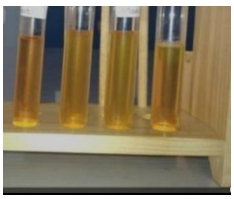
Table 3 Results from Well Water at STIkes Namira

Table 4. Results of Biochemical Reaction Tests on Well Water Samples at Namira Madina Health College

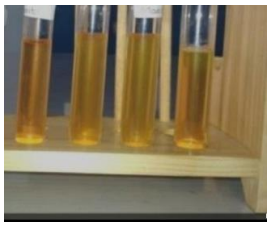
Sample Code	Candy test	Simon Citrate Test	TSIA Test	Sim Test	Urea agar test
A	+	+	+	+	+
B	+	+	+	+	+
C	+	+	+	+	+

The results of the biochemical reaction test, showed that all samples produced Escherichia coli bacteria found in each well of the water at STIkes Namira Madina, which has been contaminated because the well water can contain sugar, soil, and is close to rice fields. If the medium is yellow, it means it is able to ferment sugar (+); if the medium remains red, it means it is unable to ferment sugar (-).

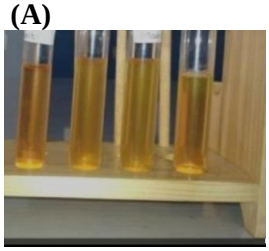
Figure 7. Results from bottled drinking water (-)



(A)



(B)



(C)

Figure 8. The results for well water A, B, and well water C at STIkes Namira Madina

In the sugar test, there was only a color change in the sugar media, which turned yellow, indicating that these bacteria formed acids from glucose fermentation. Observations were made by looking at the color change in the sugar medium; if the medium was yellow, it was marked with (+), and if it was red, it was marked with (-).

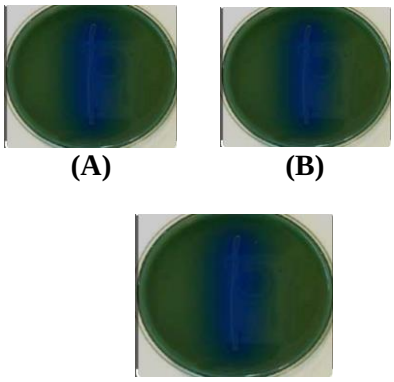
Simon Citrate Medium Test

Simon Citrate Medium is one of the media used to test the ability of bacteria to use citrate as the sole carbon source Citrate permease can transport molecules in cells and convert them enzymatically into pyruvate. Pyruvate can then be converted into various products, depending on the pH of the environment. The Escherichia coli media test will still produce green because Escherichia coli bacteria can grow by using citrate in the media as a carbon source (Lailatul Khotimah 2016).

The aim of the citrate Simon test was to determine the ability of enteric bacteria to ferment citrate into a carbon source.

In the Simon citrate medium test, one colony was grown on EMBA media on Simon citrate media in a zig-zag manner on the slanted part after incubation at 370 °C for 24 h. The results for Simon citrate are marked by a change in color from green to blue.

Figure 9. Results from bottled drinking water (-)



(A) **(B)**

(C)

Figure 10. Results from well water A, B and well water C at STIkes Namira Madina (+)

From the figure, it can be seen that the type of bacteria is seen from the observation itself, and there is a change in color in the media that is made. The results of the Simon citrate test on Escherichia coli bacteria, showed that the presence of a blue color in the middle indicates positive bacterial growth.

TSIA (Triple Sugar Iron Agar) Test Results) In Sample

The TSIA test was used to determine the ability of microorganisms to ferment sugars. The TSIA test aims to determine the physiological properties of the isolated bacterial colonies.

Colonies that grew on TSIA media by zigzagging on the surface of the slanted agar were, then punctured to the bottom of the non-slanted part. The cells were incubated in an incubator at a temperature of 370C for 24 h. Reading the TSIA results by looking at the formation of white sulfur deposits, the presence of gas medium becomes cracked, and the presence of acid fermentation means that the slanted part and the base are yellow.

4. TSIA Test Results Table

Sample Code	H2S	Gas	Slant	Deep
A	+	+	Yellow	Yellow
B	+	+	Yellow	Yellow
C	+	+	Yellow	Yellow

The test results in the table, it shows the presence of Escherichia coli bacteria from the TSIA results by looking at the formation of black sulfur deposits, the presence of gas in the medium becoming cracked, and the presence of acid/acid fermentation or (+/+), indicating that the slanted part and base are yellow. Black sulfur was not formed in the TSIA medium.

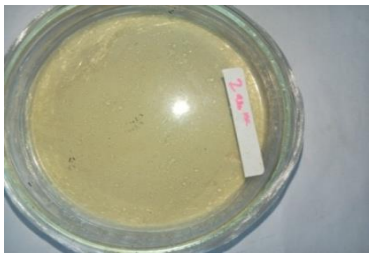
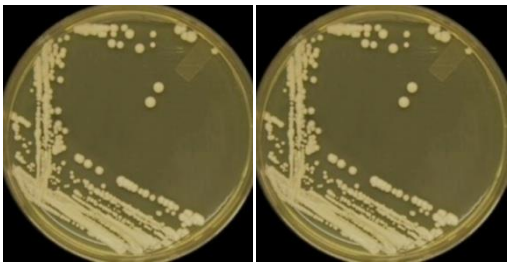


Figure 11. Results from bottled drinking water (-)

(A)



(B) **(C)**

Figure 12. Results from well water A, B and well water C at STIkes Namira Madina (+)

The results of the biochemical test on TSIA are indicated with the bottom part being yellow, as well as the slanted part, being yellow. This indicates that Escherichia coli bacteria in TSIA media can ferment glucose, maltose, lactose, and sucrose. The results of the indole test were positive after the media was dripped with Kovac's reagent, and a red ring was formed, indicating that the bacteria were moving (motile). *Escherichia coli*reading on SIM medium by looking for black sulfur deposits on the medium and presence of bacterial movement.

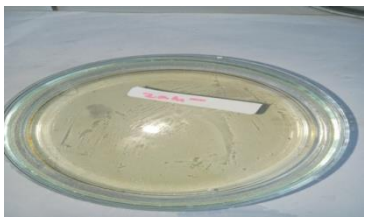
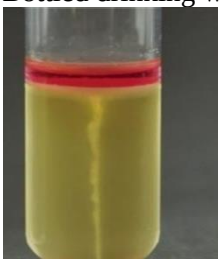
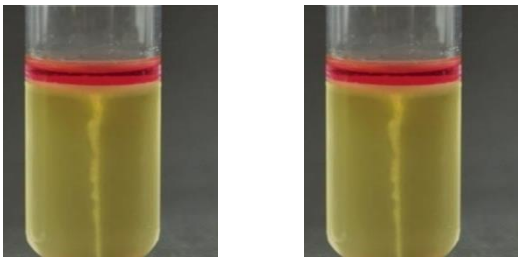


Figure 14. Bottled drinking water results



(A)



(B) **(C)**

Figure 15. Results on well water A, B and well water C at STIkes Namira Madina

The test conducted on SIM media can be observed in the indole test, which obtained results on the surface of the media that had changed to red after being dripped with Kovac's reagent, which has shown positive results, with a transparent color at the base or bottom of the media. Observations of the motility test of bacterial colonies that had previously been stabbed perpendicularly down on the media produced a white color and were shaped like an inverted pine.

Urea Agar Test Results

The urea agar test is a biochemical test that detects alkaline fermentation, which results in the production of ammonia by microorganisms.

The urea agar test aims to test the ability of an organism to produce a urea enzyme that hydrolyzes urea.

Colonies grown on EMBA medium were cultured on urea medium in a zigzag manner on a slanted medium and, then incubated at 370C for 24 h. Readers on this urea medium are (+) indicated by a change in the medium to pink and (-) indicated by a yellow-orange medium.

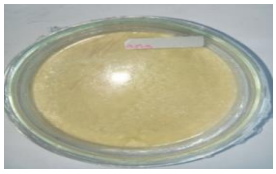
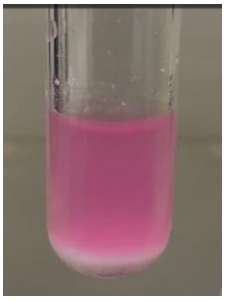
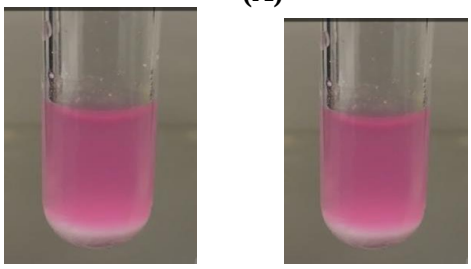


Figure 16. Results from bottled drinking water (-)



(A)



(B) **(C)**

Figure 15 The results of well water A, B, and well water C at STIkes Namira Madina (+).

Stated in Asiska Permata Dewi (2021). The results of the urea agar test on Escherichia coli bacteria on EMBA media cultured on urea agar media showed positive results with yellow media and changed color to pink. The results of the urea agar test using Escherichia coli bacteria were positive for urea production. The results of the urea agar test on Escherichia coli showed that the media changed color to pink, and the results obtained were positive. The well water at STIKes Namira Madina has been polluted because it is close to a septic tank, rice fields, and soil.

Discussion

From the bacterial identification test, where from 3 samples tested, namely sample A, B, and C, showed positive results for Escherichia coli bacteria because they were contaminated from the soil around the well, near the septic tank, and near the rice fields. This is indicated by a change in color from the original purple to metallic green.

The metallic green color indicates the presence of Escherichia coli. This is stated in the literature of Asiska Permata Dewi (2021), EMBA media contains lactose, if there are members of the genus Escherichia coli in the culture, the acid produced from the fermentation of lactose will produce a colony color that is specific to bacteria belonging to the genus Escherichia coli, namely metallic green colonies.

Next, the gram staining test, where in the three samples tested, namely samples A, B, and C, all showed positive results for Escherichia coli bacteria. This is indicated by a red to purple color. This is stated in the literature by Khairotun Nisah (2021), from the results of the gram staining test on well water under a microscope adjusted to the literature, it shows that from all samples on the glass object, all bacteria were classified as gram-negative bacteria, short rod-shaped, with round and convex colonies, and are lactose-fermenting.

Biochemical reaction tests were conducted to identify the Enterobacteriaceae family of bacteria. Biochemical reaction tests consisted of five different tests: sugar media test, Simon citrate test, triple sugar iron agar media test, sulfur indolmoltility media test, and urea agar test.

The sugar media test where in 3 samples tested in samples A, B. This is stated in the literature of Asiska Permata Dewi ddk (2021), EMBA media cultured in glucose, lactose, maltose and sucrose media showed a color change from red to yellow.

The Simon citrate media test showed positive results for Escherichia coli bacteria in three samples tested in samples A, B, and. This is stated in the literature of Andi Partunggul Pasaribu (2019), where the Simon citrate media seen in the change in media from green to blue indicates the growth of Escherichia coli bacteria.

Meanwhile, the Triple Sugar Iron Agar media test, where the three samples tested in samples A, B, and C all showed positive results for Escherichia coli bacteria. According to Asiska Permata Dewi (2021), the formation of black sulfur deposits, the presence of gas in the medium the presence of acid fermentation/acid, the slanted part of the medium is red, and the base of the medium is yellow.

Next, the sulfur indole motility media test, in which three samples tested in samples A, B, and C all showed positive results for Escherichia coli bacteria. This is stated in the literature of Khotimah (2016), who showed that Escherichia coli bacteria in TSIA media can ferment glucose, maltose, lactose, and sucrose. The results of the indole test were positive after the media was dripped with Kovac's reagent, and a red ring was formed, indicating that the bacteria were moving (motile).

Next, the Sulfur Indol Moltility media test, where the three samples tested in samples A, B, and C all showed positive results for Escherichia coli bacteria. This is stated in the literature by Asiska Permata Dewi (2021), which can be seen by observeing the presence of black sulfur deposits in the medium, the movement of bacteria by seeing white fog on the puncture marks into the medium, and the formation of a red ring after being dripped with Kovac's reagent, indicating the formation of indole.

The urea media test showed positive results for Escherichia coli bacteria in three samples tested in samples A, B, and. This is stated in the literature of Asiska Permata Dewi (2021), colonies that grow on EMBA media are cultured on urea agar media in a zigzag manner on slanted media, on positive urea media it is indicated by a change in color from pink to orange yellow.

CONCLUSION

Bottled drinking water does not contain Escherichia coli bacteria in selective media, namely, EMBA. Well water at STIkes Namira Madina Panyabungan contains Escherichia coli bacteria in selective media, namely, EMBA. The quality of bottled drinking water sold around Panyabungantimur is suitable for consumption based on regulations that meet the mandatory quality requirements of the Indonesian National Standard (SNI) and can be distributed by the Food and Drug Supervisory Agency (BPOM). because there were no Escherichia coli bacteria in the results of the study.

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