Research Article

Microbiological Analysis of Drinking Water Sourcing from the Spring of Joben Pesanggrahan, Montong Gading, East Lombok

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Abstract | Water is an essential component for humans, mainly used as drinking water. There are still many people in Indonesia who use springs as a source of drinking water, one of which is in Joben, Pesanggrahan Village, Montong Gading District, East Lombok Regency. Drinking water from springs can be polluted by contaminants such as bacteria, viruses and others during storage and distribution. This study aims to analyze the quality and microbiological feasibility of drinking water sourced from Joben Springs, Montong Gading, East Lombok. The research was carried out in May-June 2021. The research method used was purposive sampling with sampling carried out at three points: the spring, the main reservoir, and the residents' reservoir. The Coliform test was carried out using the Most Probable Number (MPN) method and *Escherichia coli* (*E. coli*) identification using Kirby Bauer. The results of this study indicate that the coliform MPN test from the spring sample obtained the highest value in the paddy field, spring, and house sample of 4,050/100 ml and was classified as class E clean water. Moreover, it was very poor water; the lowest was in mountain storage water of 390/100 ml, which was classified as water clean class C bad category. Water from the Joben spring is not suitable for direct consumption by the community, so it needs to be boiled first to kill bacteria and minimize health problems. All samples, there were no coliform bacteria of the *E. coli* group. The bacteria in all samples were bacteria from other coliform groups. Therefore, further analysis is needed to determine the content of other bacteria in the water at the Joben spring, Pesanggrahan Montong Gading, East Lombok.

Keywords | Drinking Water, Bacteria, Joben's Springs, Coliform, Pesanggrahan

Received | January 26, 2023; **Accepted** | March 17, 2023; **Published** | April 01, 2023
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Citation | Jupri A, Vofi Y, Faturrahman, Rohyani IS, Ernawati, Bulkaini, Kiswowo D, Jannah W (2023). Microbiological analysis of drinking water sourcing from the spring of joben pesanggrahan, montong gading, east lombok. Anim. Vet. Sci. 11(5): 756-763.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.5.756.763
ISSN (Online) | 2307-8316

INTRODUCTION

Water is very important for humans, approximately 65% of the total human body weight is water and this volume varies significantly for each person (Chandra and Budiman. 2007). The human body is composed of millions of cells and almost the entire cell contains water (H_2O) (Yusuf, 2002).

Humans need water for various purposes, such as bathing, cooking, and most importantly for everyday consumption (Sunarti, Riri., 2016). Water is essential to life and the principal inorganic constituent of living matter, generally making up nearly three-quarters of the weight of a living cell. Water serves as a second natural medium for the growth of microorganisms (Ezemba et al., 2021).

The main benefit of water for humans is drinking water. Drinking water can come from rainwater, surface water, groundwater, and springs. Safe drinking water is essential to prevent the spread of waterborne diseases. Safe drinking water is defined as water that does not pose a significant risk to health during consumption (Fewtrell and Colford, 2005). Standards for in Indonesia are stipulated by a Regulation of the Minister of Health of the Republic of Indonesia Number: 492/MENKES/PER/IV/2010, which contains requirements for potable water (Peraturan Menteri Kesehatan, 2010).

Drinking water can be polluted at sources, distribution channels, and/or at the household level, and this polluted water can become a transport medium for several pathogens (Chandra and Budiman, 2007). One of the microorganisms that contaminate water is coliforms. Coliforms are gram-negative rod-shaped bacteria that are facultative anaerobes, can ferment lactose to produce gas at temperatures of 35^0C to 37^0C, and do not form spores. Coliform is usually used as an indicator in determining water quality (Tominaga, 2019; Fatemeh et al., 2014). The presence of coliform in water indicates environmental pollution and the presence of waterborne disease bacteria (Nicholson et al., 2016). Coliform bacteria also make it possible to assess the efficiency of water treatment (disinfection, chlorination, or boiling), so their presence indicates insufficient, inadequate, or non-existent water treatment (Berg et al., 1978). Groundwater has become the safest and most abundant source of potable water in comparison to surface water as it is often shielded from direct human activities. However, pollution of groundwater resources can occur directly from municipal wastewater, industrial discharges, agricultural waste, urban runoff, landfills, or waste dumps and indirectly from air pollution (Adebayo et al., 2015). Many studies have reported the results of interventions to reduce illness through improvements in drinking water, sanitation facilities, and hygiene practices in less developed countries. There has, however, been no formal systematic review and meta-analysis comparing the evidence of the relative effectiveness of these interventions (Fewtrell and Colford JM (2005).

The amount of coliform in the environment is influenced by many factors, including rainfall and turbidity. Adequate food availability can help coliforms reproduce and grow to form colonies in a dark, warm, and humid environment (Wang and Deng, 2019). Coliform bacteria can be divided into two groups: fecal coliform and non-fecal coliform. One example of fecal coliform is Escherichia coli (*E. coli*), a bacterium from animal and human feces (Suriawira, 1996). *E. coli* detection is an essential indicator of the presence of pathogens (disease-causing organisms such as bacteria, protozoa, and viruses) in drinking water (Ibrahim, 2019; Meride & Ayenew, 2016). The UN-Water Global Assessment and Analysis of Sanitation and Drinking Water 2019 (known as the GLAAS report) surveyed 115 countries and territories, representing 4.5 billion people. It showed that, in an overwhelming majority of countries, the implementation of water, sanitation, and hygiene policies and plans is constrained by inadequate human and financial resources. Nineteen countries and one territory reported a funding gap of more than 60% between identified needs and available funding. Less than 15% of countries have the financial or human resources needed to implement their plans. (WHO, 2019).

The pathogenic bacteria usually found in contaminated waters are Salmonella, Shigella sp, Vibrio cholera, and *E. coli* (Lestari and Hanani, 2015). Various diseases caused by coliform bacteria, such as respiratory tract infections, digestion (typhoid and paratyphoid fever), dysentery (bacillus and amoebic), diarrhea, hepatitis A virus, and cholera (Hasanuddin, 2013; Adebayo et al., 2015). Therefore, the WHO determines that domestic water standards do not contain total coliform and *E. coli* (Meride & Ayenew, 2016).

Sources of drinking water in several regions in Indonesia are still sourced from springs. One of them is in Joben, Pesanggrahan Village, Montong Gading District, East Lombok Regency. The community usually directly consumes water sourced from the Joben spring without boiling it. The community usually consumes water sourced from the Joben spring directly without boiling it first. It is suspected that the water quality from the Joben spring has become polluted. Consumption of water directly can cause health problems for the community, such as diarrhea, skin diseases (itching), and others.

The incidence of diarrhea caused by bacteriological content in drinking water is quite high in the East Lombok area. Based on data from the East Lombok Health Office in 2015 the discovery and treatment of diarrhea was 101.615% (50,623 people). The results of a survey by the authors at the Montong Betok Health Center found data on the ten most common diseases treated at the RRI (Inpatient Room) in December 2017; diarrhea was in third place out of the ten most common diseases after Gastritis and Febris.

Several studies have been conducted to test the quality of drinking water in Lombok. The results of Pomalingo's research (2012) on the bacteriological quality of drinking water sourced from springs in North Bilungala Village, Bone Pantai District, Bone Bolango Regency, showed the presence of coliform and *Escherichia coli* bacteria in several research samples that did not meet health standards in the

bad drinking water category. Widiyanti (2019) conducted research related to testing the content of *E. Coli* bacteria in groundwater in Dasan Lekong Village using the MPN (Most Probable Number) method, showing the results that all dug wells which were sampling locations were contaminated with *E. coli* bacteria with a value range of 490 to more than 24000 per 100 ml of well water. Contamination of well water by *E. coli* bacteria is related to pollutant sources such as septic tanks, the distance between wells and pollutant sources, landfills, and inadequate sanitation facilities. Mahendra et al. (2022) showed that the Mumbul Sari spring water in North Lombok Regency was contaminated with coliform and *E. coli* bacteria with values exceeding the quality standards set by the Ministry of Health of the Republic of Indonesia. The MPN coliform value of spring water used by women is higher than that of spring water used by men (300 CFU/100ml > 250 CFU/100 ml). Likewise, the number of *E. coli* bacteria (50 CFU/100ml > 12 CFU/100ml).

Based on these problems, testing the quality of drinking water is very important to identify the contaminants in the water to process and prevent health hazards. Analysis of water quality in Joben springs has never been done before. This research was conducted to analyze the quality and microbiological feasibility of drinking water sourced from Joben Springs, Montong Gading, East Lombok.

Methodology

The research was conducted at Joben Springs, Pesanggrahan Village, Montong Gading District, East Lombok Regency, in May-June 2021. Sampling was carried out at three points, namely at the spring, in the primary storage tank and the resident's house holding tank. Sample testing was carried out at the UPTD Health Laboratory for Calibration Testing and Medical Support in West Nusa Tenggara Province.

This research is experimental in nature, using the method of determining the value of the MPN to know how to analyze the water in the sample quantitatively.

Research Tools and Materials

The tools and materials in this study were laboratory glassware, BSC (Biological Safety Cabinet), binocular microscope, 37°C incubator, spring water samples, Lactose Broth (LB) consisting of Triple Strength Lactose Broth (TSLB) and Single Strength Lactose Broth (SSLB), diluent (phosphate buffer), Brilliant Green Lactose Broth (BGLB) media, Brain Heart Infusion (BHI), Eosin Methylene Blue Agar (EMBA), indole reagent, urea, TSIA, simmons citrate, lugol, safranin, crystal violet , glucose, and sucrose.

Research Procedure

This research was conducted in two stages: sampling and testing for coliform bacteria and *E. coli.*

Sampling Stage

The sampling of drinking water sourced from the Joben spring, Pesanggrahan, Montong Gading District, East Lombok Regency used sterilized glass bottles. Testing of water samples was further analyzed at the UPTD Health Laboratory for Calibration Testing and Medical Support in West Nusa Tenggara Province.

Testing for *Coliform* Bacteria

Presumptive Test: The presumptive test was carried out using a 5 5 5 variance, which consisted of 5 x 10 ml, 5 x 1 ml and 5 x 0.1 ml variances. At this stage, 20 tubes were prepared, equipped with Durham tubes. In the first five tubes, 10 ml of TSLB medium was added, and 10 ml of water sample was inoculated into the fifth series of tubes. Then, 5 ml of SSLB medium was put in, and 1 ml of water sample was inoculated. Next, the water sample was diluted by dissolving 1 ml of water in a bottle containing 9 ml of Buffer Phosphate solution and shaking it until homogeneous. The second dilution was carried out by taking 1 ml of the first dilution and inoculating it into 9 ml of phosphate buffer. In the five series of the third tube, put 5 ml of SSLB medium and inoculate 1 ml of the dilution using a sterile measuring pipette. In the five series of the fourth tube, 5 ml of SSLB medium was put in, and 1 ml of the result of the second dilution was inoculated using a sterile pipette. Then the test tube rack was shaken gently, and the sample was spread evenly over all parts of the media. Then incubated at 37°C for 1-2 x 24 hours and observed the formation of gas (air bubbles in the Durham tube) and acid (the medium became cloudy).

Coliform Confirmatory Test: The Coliform Confirmation Test was carried out by preparing culture tubes containing 10 ml of Brilliant Green Lactose Broth (BGLB) media equipped with a Durham tube. Then 1 to 3 oses were inoculated from each tube that was positive in the presumptive test into the BGLB medium. Then the culture tube was incubated at 37°C for 1-2×24 hours.

***E. coli* Testing:** The *E. coli* test was carried out by inoculating 10 ml of the sample into 90 ml of BHI media. Then incubated at 37°C for 24 hours and observed the color change in the medium. Then the positive samples were inoculated as much as 1 or 2 oses onto the surface of the EMBA medium in a zigzag manner and then incubated at 37°C for 24 hours. Colonies that show metallic green on Eosin Methylene Blue (EMB) media are colonies of *E. coli* bacteria. Followed by a gram and chemical test consisting of IMViC and sugar tests.

Gram test (cell shape and arrangement).

Positive results shown on EMBA media can be followed by gram staining to differentiate gram-positive and gram-negative types. Gram staining begins with the preparation of the bacteria used and air-dried. The dried preparations were given 1 drop of crystal violet solution which was left for one minute and rinsed with running water. Then, Lugol's solution was left for 1 minute and rinsed with running water. After that, add alcohol until the color of the preparation disappears and add safranin for 15 seconds and rinse with running water. The next process is drying, followed by observation with a microscope with a magnification of 100 times.

Biochemical test with sugar test and IMViC: Indole test. The EMBA culture was planted in 1 ose into the tryptone broth and incubated for 24 hours at 37°C. After that, 3-5 drops of indole reagent were added. Homogenize and then let stand for a few minutes. The indole test will show a positive result if the solution contains a red ring.

Citrate Test: The EMBA culture was planted 1 ose into Simmons citrate and incubated for 24 hours at 37°C. The citrate test will show a positive result if a color changes from green to blue.

TSIA test: TSIA test. The EMBA culture was grown one ose into the TSIA and incubated for 24 hours at 37°C. The TSIA test will show a positive result if it produces acid.
Tests for sugars include the Glucose and Sucrose test. One ose of the bacterial isolates in EMBA was inoculated into test tubes containing glucose and sucrose and incubated for 24 hours at 37°C. A change indicates a positive test in the color of the medium to yellow; if there are bubbles in the tube, the fermentation produces gas (CO_2). A negative test is indicated by the color of the medium not changing.

RESULTS AND DISCUSSION

Coliform content analysis in this study used the MPN method. A confirmation test is carried out to ensure the presence of Coliform bacteria because, in the prediction test, positive results are not always caused by the presence of Coliform bacteria. Positive test results can also be caused by other bacteria that can ferment lactose accompanied by the formation of gas and acid. In the confirmation test, a selective medium was used, namely BGLB media containing bile salts which can inhibit the growth of *gram-positive* bacteria that do not live in the human digestive tract and contains brilliant green which can inhibit the growth of certain *gram-negative* bacteria other than coliform (Winasari, 2015). Positive results for the coliform test were indicated by turbidity and gas bubbles in the Durham tube in a test tube containing BGLB media within 48 hours (Figure 1).

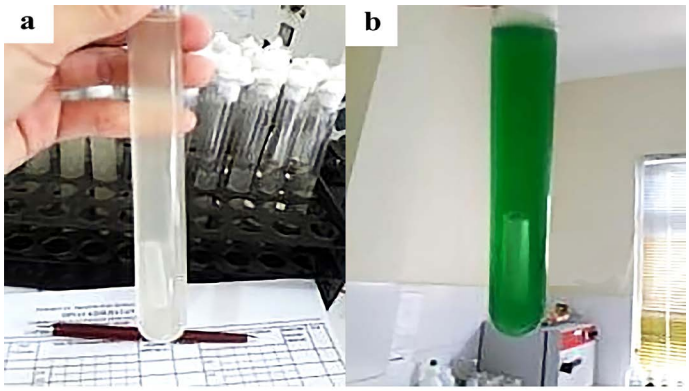

Figure 1: Positive test (a) preliminaries and (b) confirmation.

The color change is caused by bacteria producing acid and the formation of bubbles and is related to coliform's ability to ferment the lactose (Rojas et al., 2020). The formation of bubbles indicates that a lactose fermentation process has occurred and is an indicator of the growth of coliform bacteria which is the basis for determining the coliform MPN value (Saridewi et al., 2016).

Table 1 shows that the samples tested from the affirmation test results had the highest Coliform MPN values in paddy fields of 4,050/100 ml. In contrast, the lowest MPN Coliform value was found in a mountain shelter, 390/100 mL. The results of the observation of the affirmation test based on the Decree of the Minister of Health of the Republic of Indonesia Number: 492/MENKES/PER/IV/2010 that all drinking water samples did not meet the requirements for the maximum limit for total coliform bacteria and were unfit for consumption. It is because coliform bacteria were used as indicator bacteria for the presence of pathogenic bacteria.

Based on the research results, it is known that the highest MPN value is in paddy field springs compared to mountain springs. The high MPN coliform value in paddy field springs can be affected because the springs are open without a reservoir and the location of the water source is between the paddy fields and settlements. It allows the soil and water to be contaminated by residue from human activities such as household and agricultural waste. In addition, the piping system for distributing water from springs to holding tanks through rice field ditches is made of mossy cement without any cover. It also allows contaminants from outside to enter the water stream.

Coliform MPN values in mountain springs are low because water reservoirs are slightly closed compared to the reservoirs in rice field springs. Construction of storage

Table 1: BGLB Affirmation Test Results (*Brilliant Green Lactose Broth*).

No	Sampling Point	Result from MPN/100 mL	Quality Standard Score (ABM)	Interpretation
1	Paddy Field Springs	>1600	0/100 mL	Not fit for consumption
2	Paddy Shelter	2.550	0/100 mL	Not fit for consumption
3	Paddy House	4.050	0/100 mL	Not fit for consumption
4	Mountain Springs	1.030	0/100 mL	Not fit for consumption
5	Mountain Shelter	390	0/100 mL	Not fit for consumption
6	Mountain House	551	0/100 mL	Not fit for consumption

tanks made of cement and pipe flow through settlements, roads and rice fields. According to Suriawiria (1996), the types of pollutants that enter water bodies come from domestic sources (households, villages, market towns and roads) and non-domestic sources (factories, industry, agriculture, animal husbandry and fisheries).

Based on the Decree of the Director General of PPM and PLP No.1/PO.03.04.PA.91 and the Decree of JUKLAK on Water Quality Guidelines 2000/2001, the results of analysis on the paddy field and house water samples were classified as class E clean water, very bad category. It contains more than 2400 coliform, while the results of samples of mountain springs and paddy field springs were classified as class D clean water; the very poor category contained coliform 1001-2400. Furthermore, the results of samples of mountain shelters and mountain houses were classified as class C clean water, the poor category containing coliform 101-1000.

E. coli can grow well at temperatures between 20°C-45°C, whereas at temperatures below 4°C, *E. coli* will experience a dormancy or sleep phase. *E. coli* can die at temperatures above 50°C within 10 minutes (Sunarko, 2012). The presence of E coli bacteria was tested using Brain Heart Infusion (BHI) media. BHI is a fertilizer medium for the growth of various types of bacteria, both in liquid and agar form. Turbid media is inoculated on EMBA media, and benthic media is selective in growing *E. coli* because the media contains eosin which can inhibit the growth of *gram-positive* bacteria and can only grow *gram-negative* bacteria. If the culture contains *E. coli* bacteria, the acid produced from the fermentation will produce a specific colony color for *E. coli* bacteria: metallic green colonies.

Table 2 shows the results of inoculation on EMBA media which produced metallic green colonies in the rice house samples. According to Mahon (2015), *E. coli* bacteria can ferment lactose quickly and produce much acid to produce shiny metallic colonies with metallic green pigment deposits. The results of observations also found colonies of pink and purplish pink (Figure 2). Ryan and Ray (2014) state that other bacteria could grow on EMBA media: the Enterobacteriaceae family, for example, Klebsiella sp., Enterobacter aerogenes, and Pseudomonas aeruginosa.

Table 2: Observation results of EMBA colony morphology.

Sample	Shape	Color
Paddy Field Springs	Irregular and Round	Pink, Purplish Pink
Paddy Shelter	Irregular and Round	Pink, Purplish Pink
Paddy House	Irregular and Round	Metallic Green, Pink, Purplish Pink
Mountain Springs	Irregular and Round	Pink, Purplish Pink
Mountain Shelter	Irregular and Round	Pink, Purplish Pink
Mountain House	Irregular and Round	Pink, Purplish Pink

The Gram stain results showed that the samples suspected to be *E. coli* were red, and the short rods were pink. It is because the sample has a cell wall composition that contains more lipopolysaccharide than the gram-positive bacteria group, so these bacteria do not retain the crystal violet substance. When stained with safranin, the bacteria will retain the safranin color, which is a pink color (Figure 3).

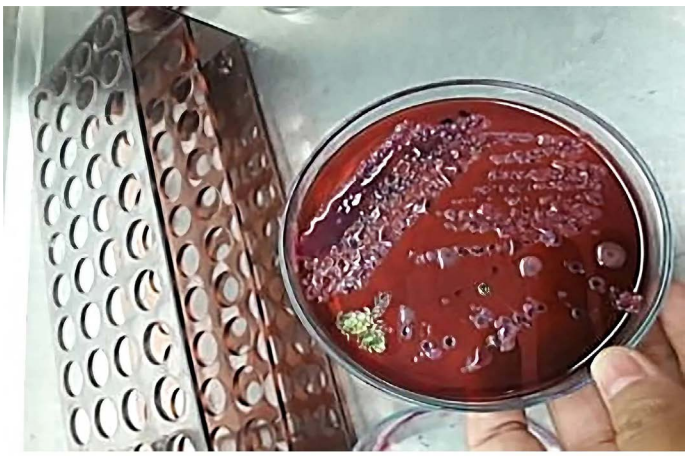

Figure 2: Samples on EMBA Medi Turn Metallic Green.

Table 3: Results of Spring Sample Completeness Test.

No	Sampling Point	Biochemical Results Sampling Point						Interpretation
		TSIA	S.St	GI	Sk	Ur	In	
1	Paddy Field Springs	-	-	-	-	-	-	
2	Paddy Shelter	-	-	-	-	-	-	
3	Paddy House	+	+	+	+	-	+	Further testing is carried out
4	Mountain Springs	-	-	-	-	-	-	
5	Mountain Shelter	-	-	-	-	-	-	
6	Mountain House	-	-	-	-	-	-	

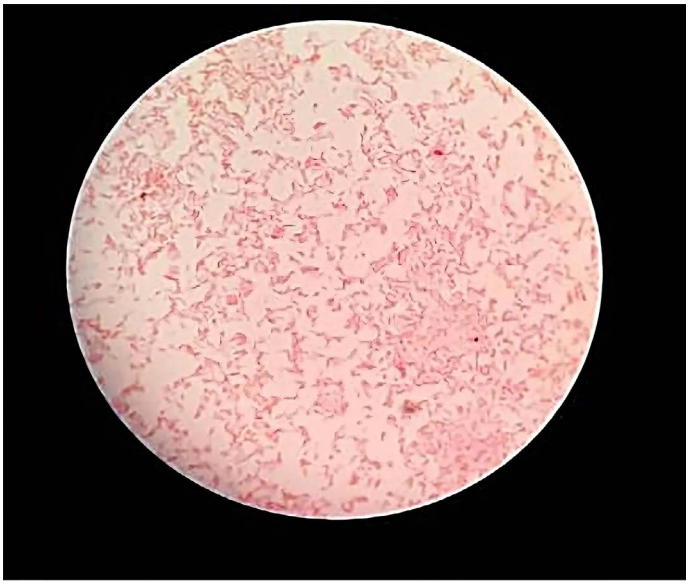

Figure 3: Gram Stain Results

The results of the TSIA test were that the bottom and slanted parts were yellow, indicating an acidic atmosphere at the bottom and slant (Figure 4 (a)). Following Lebofee (2011), the results of the TSIA test on *E. coli* produce a yellow color because *E. coli* in TSIA media can ferment glucose, lactose, and sucrose.

The citrate test obtained positive results; the bottom is green, and the bag is blue. However, this is different from the theory that the result of the citrate test for *E. coli* bacteria is negative because *E. coli* cannot use citrate as a carbon source. This test shows the ability of bacteria to use citrate as the only carbon source. If the bacteria can use citrate as a carbon source, it will raise the pH and change the color of the culture medium from green to blue (Bambang, 2014) (Figure 4 (b)).

The sugar test results obtained positive results for glucose and sucrose media which were marked by a color change from red to yellow accompanied by gas formation. According to Harley (2012) the color change of the media to yellow is due to the presence of the phenol red indicator due to the formation of acid in the sugar test medium. This sugar test aims to see the ability of microorganisms to ferment these sugars, and the results of this test follow the *E. coli* bacteria test, which is positive for the sucrose and glucose tests (Figure 4 (c) and Figure 4 (d)).

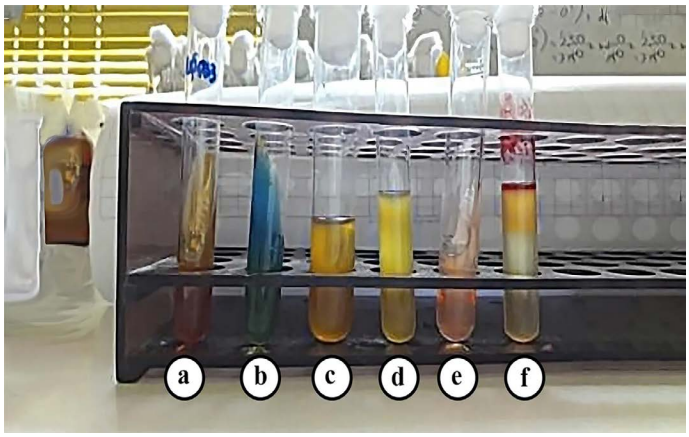

Figure 4: Biochemical test result successive TSIA test (a), Simon Citrate Test (b), GLucose test (c), Sucrose test (d), Urea test (e), Indole test (f).

The urea test obtained a negative result where there was no color change in the medium, which remained orange. The color of the media that changes from yellow to pink indicates that the bacteria have the enzyme urease (Brown, 2011). According to Leboffe and Pierre (2011), positive results are indicated by a color change from orange to red, while negative results indicate no color change in the media. The results of this test follow the positive test results for *E. coli*; no color change in the urea media (negative) (Figure 4 (e)).

The indole test obtained a positive result indicated by the formation of a red ring on the surface of the culture. The results of the Indole test on *E. coli* bacteria were positive, indicating a red ring at the top because the indole reacted with aldehydes (Rahayu, 2017) (Figure 4 (f)) (Table 3).

The results of several biochemical tests above found no *E. coli* bacteria in all water samples from the Joben Springs, Pesanggrahan, Montong Gading Subdistrict, East Lombok Regency. It was suspected that the bacteria in the water samples were from other groups of Coliform bacteria.

ACKNOWLEDGMENTS

The authors wish to thank the Pesanggrahan village government for providing research grants. The authors also thank the Montong Betok Health Center cooperating in the data collection process. Lastly, the authors would like to thank the microbiology laboratory team of the Faculty of Mathematics and Natural Sciences, University of Mataramand and the Food and Drug Supervisory Agency (BPOM) for helping to measure research variables.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELTY STATEMENT

This research was conducted for the first time and found that the Joben spring used as drinking water by the people of Pesanggrahan Village contains coliform bacteria and is not qualified for direct consumption.

AUTHOR'S CONTRIBUTION

The research process and data collection are entirely the responsibility of Ahmad Jupri and Yuliana Vofi. The research data was processed by Faturrahman and Immy Suci Rohyani, while the manuscript was written and edited by Ernawati, Bulkaini, Djoko Kisworo and Wardatul Jannah. Collectively revise the substance of the paper so that it is worthy of publication.

CONCLUSIONS AND RECOMMENDATIONS

This research concludes that all positive samples contained coliform, with the highest coliform value obtained in a sample of paddy spring water of 4,050/100ml and was classified as class E clean water, very bad category. Moreover, the lowest coliform value was obtained in a sample of mountain water storage 390/100ml and is classified as class C water in the bad category. In all samples, there were no coliform bacteria of the *E. coli* group. Water from the Joben spring is not suitable for direct consumption by the community, so it needs to be boiled first to kill bacteria and minimize health problems. The results showed no coliform bacteria belonging to the *E. coli* class. The bacteria present in all samples were thought to be bacteria from other coliform groups. Therefore, further analysis is needed to determine the content of other bacteria in the water in the Joben spring.

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