The Overview of Microbiological Quality of the Upstream of Cikapundung River Based on Total Coliform and Escherichia coli

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Abstract. Cikapundung River is one of many rivers which its existence used by Bandung citizens for many function. One of the function is main water source for Dago Pakar Water Treatment Plant which is located in Bantar Awi. The social activity as a cow farmer can contaminate the quality of Cikapundung River because of the disposal of the waste that didn't go through the process. The purpose of this research was to determine the amount of Total Coliform Bacteria and Escherichia coli contained in the upstream of Cikapundung River and to adapt with the regulation of the minister of the health of the Republic of Indonesia Number 32 of 2017. This research is a descriptive study conducted at Microbiology Laboratory, Faculty of Medicine in Jenderal Achmad Yani University. The sample was collected from three spots: upstream, midstream and downstream on three times which are morning, daylight, and afternoon. The method used is Most Probable Number with three steps including Presumptive test using Lactose Broth, Confirmed Test using BGLB and Completed Test using EMBA. The presumptive test showed all samples are positive except for the first spot which is from the upstream as main water source. The confirmative test showed on second and third spot taken on morning, daylight and afternoon contain very high amount of coliform which is 1101 CFU/100 ml. On completed test, E. coli, Enterobacter and Klebsiella colonies were found. It can be concluded that microbiological quality of Cikapundung River is not yet fulfilled the standard.

Keywords: Cikapundung River · Coliform · Most probable number

1 Introduction

Water is a natural resource that has been given by nature that is needed by living things all the time. Water that has poor quality will result in bad conditions for human survival so that it will affect human health conditions [1]. According to the Regulation of the Minister of Health of Indonesia Number 32 of 2017, water can be said good for health if there

are no Escherichia coli and Coliform Bacteria (max 50) found on 100 ml water sample [2]. Contaminated water can become the transmission media for coliform bacteria [3]. Coliform can be classified into two groups, which are fecal coliform such as Escherichia coli and non-fecal coliform such as Klebsiella and Aerobacter [4]. In Indonesia, rivers have been used as a means for drinking water and irrigation facilities for agriculture and fish farming so far. The city of Bandung has two rivers as its main flow, namely the Cikapundung River and the Citarum River [5]. One of the rivers used for its existence is the Cikapundung River. Cikapundung River has 28 km length and flows over Bandung City, Bandung Regency, and West Bandung Regency. On the upstream of Cikapundung live approximately 7.000 stock farmers, who have in average 2–3 cows, and just a few big farmers who have more than 1000 cows. Most of the farmers are disposing to the river. The river is used for Drainage canal, tourism, agricultural irrigation facilities, water power plant, Water source for Bandung Municipal Supply Company (PDAM) and Drinking Water Treatment Plant (IPAM) Dago Pakar Bantar Awi.

Based on the inspection of the raw water quality of the Cikapundung River conducted in April, June, September, December 2012 and March 2013, there were 12 parameters that did not meet the requirements, including Total Coliform and Fecal Coliform. Regarding "Description of Microbiological Quality of Water from the Upper Cikapundung River Based on the Total Number of Coliforms and Escherichia coli" to determine the microbiological quality of water caused by various activities of the surrounding community.

2 Materials and Methods

This study used a descriptive research design to describe the microbiological quality of water in the upstream of the Cikapundung River. The method used in this research is the Most Probable Number method.

2.1 Research Objects

The object of this research are water samples from three points of upstream flow of the Cikapundung River. Sampling was carried out at three times, in the morning, daytime and afternoon. The collection at that time was carried out because in the morning and evening the farmers cleaned the drums so that the waste would be discharged into the Cikapundung River. Daytime sampling was used as the time of no activity and spot 1 as spring or control.

The inclusion criteria for this study were that the water sample came from the upstream of the Cikapundung River at 3 points of collection, and the water sample was taken at a predetermined time. The exclusion criteria for this study were water samples from an unspecified time, and water that was not taken from a predetermined point.

2.2 Number of Samples

The number of samples came from the water of the Cikapundung River in the Bantar Awi area, namely 3 samples in 2 times of collection, so that in total there were 7 samples with 3 repetitions each (triplo). The number of water samples per point was taken 100 ml.

2.3   Place and Time of Research

This study was conducted at the Microbiology Laboratory of Jenderal Achmad Yani University, Faculty of Medicine starting from September 2021 until January 2022.

2.4   Research Procedure

The research was started by taking samples in the form of water from the midpoint taken from each predetermined location. Samples were taken using a sterile tube. Each tube is marked/labeled by including the point and time as a differentiator. Sampling was carried out at different times because the samples were taken at the time after the livestock activity and at the time when there was no activity. The sample is tightly closed and put into a box that has been designed so that the sample does not break or spill during transportation from the field to the laboratory.

2.5   MPN Step I Presumptive Test

One milliliter of the sample was transferred with a sterile pipette into 9 ml of 0.1% Butterfield Phosphate Diluent to obtain a 10^{-1} dilution solution. 1 ml of the 10^{-1} dilution solution was transferred with a sterile pipette into 9 ml of 0.1% Butterfield Phosphate Diluent to obtain a 10^{-2} dilution, in the same way as above made a 10^{-3} dilution. Each 1 ml of each dilution was taken with a pipette, then put into 3 series of tubes containing Lactose Broth Single Strength (LBSS) and Lactose Broth Double Strength (LBDS) media containing Durham tubes. The tube is then incubated at a temperature of 35–37 °C for 24 h to 48 h. Note the gas that forms in the Durham tube. The test results are declared positive if gas is formed.

2.6   MPN Step II Confirmed Test

Prepare a tube containing 10 ml of Brilliant Green Lactose Bile (BGLB) broth with a Durham tube in it. Insert one review of inoculum from each tube of LST broth that produces a positive test into each tube of BGLB broth. Incubate all tubes at 35–37 °C for 48 h. Interpretation of a positive result if the media is cloudy and gas is formed (must be both). Interpretation of negative results if there is no growth no gas is formed. Determine the amount of Coliform (MPN/g or ml) by counting the positive tube then match it to the MPN table.

2.7   MPN Step III Completed Test

The positive tube containing gas from the BGBB medium was taken and then streaked into the medium and incubated at 35–37 °C for 18–24 h. Interpret as suspected E. coli if it has flat colony characteristics, black in the center of the colony with or without metallic sheen.

2.8   Data Analysis

Based on the results of microbiological examination, data obtained on the average number of E. coli and Coliform in water samples upstream of the Cikapundung River from 3 points out of 2 collection times.

2.9   Research Ethics

This research was approved by the Health Research Ethics Commission of the Faculty of Medicine, Jenderal Achmad Yani University with ethical approval number 024/UH1.11/2021.

2.10   Research Ethical Aspects

This research was approved by the Health Research Ethics Commission of the Faculty of Medicine, Jenderal Achmad Yani University with ethical approval number 056/UM2.11/2021.

3   Results and Discussion

Research has been carried out on samples of upstream water from the Cikapundung River using the Most Probable Number method from three points, namely spot 1 which is a spring in Sunten Jaya Village located in Buleng, spot 2 is located at the meeting point of the drainages of Pasir Angling and Cikapundung, Spot 3 is located at Behind the Lodge Maribaya. Each sample was taken in the morning, daylight and afternoon except for sample point 1 which was only carried out once during the day because that point was the control. A total of 7 samples were tested to determine the content of coliform bacteria using the Most Probable Number method.

3.1   Presumptive Test Results

Based on the results of the study in Table 1, the positive results of this test can be seen by the presence of gas bubbles in the Durham tube that has been incubated. The results of the presumptive test showed positive results in all tubes, both LBSS and LBDS, except for the spot 1 daylight. At spot 1 no pollution was found because this point is a water source so it does not get direct contamination either from farms or from the surrounding community (See Fig. 1).

3.2   Confirmed Test Results

Based on the results of the study in Table 2, this confirmed test also shows the same positive results as the previous test. To interpret the MPN from the number of coliforms in each sample, the MPN table series 3 is used based on a combination of positive BGLB tubes. Based on the calculation using the MPN formula with a series of 3 tubes, the total MPN was 1101 CFU/100 ml in all samples in the morning, afternoon and evening.

Table 1. Presumptive test results

Sample	0,1 ml	1 ml	10 ml	Total positives
Spot 2 morning	+++	+++	+++	3-3-3
Spot 3 morning	+++	+++	+++	3-3-3
Spot 1 daylight	---	---	---	0-0-0
Spot 2 daylight	+++	+++	+++	3-3-3
Spot 3 daylight	+++	+++	+++	3-3-3
Spot 2 Afternoon	+++	+++	+++	3-3-3
Spot 3 afternoon	+++	+++	+++	3-3-3

Fig. 1. Presumptive test results.

Table 2. Confirmed test results

Sample	0,1 ml	1 ml	10 ml	Total positives
Spot 2 morning	+++	+++	+++	3-3-3
Spot 3 morning	+++	+++	+++	3-3-3
Spot 2 daylight	+++	+++	+++	3-3-3
Spot 3 daylight	+++	+++	+++	3-3-3
Spot 2 Afternoon	+++	+++	+++	3-3-3
Spot 3 afternoon	+++	+++	+++	3-3-3

Fig. 2. Confirmed test results.

These results indicate that the sample does not meet the microbiological parameter requirements based on the Minister of Health's Regulation, even the number is very far beyond the limit, where the standard parameter for the total coliform bacteria in river water is 50 CFU/100 ml.

This shows that the research that has been carried out still exceeds the standards of the Minister of Health Regulation for hygiene and sanitation quality standards. High levels of coliform bacteria are caused by several factors that can affect, including sanitation that can affect river water such as direct waste disposal into rivers, or the influence of farm animals themselves such as health and feed. During these hours, milking activities and cleaning of drums are being carried out. Other factors can also be caused by the surrounding community such as throwing human waste directly into the river. Topographic factors can also affect the arise of bacteria, especially at spot 2 which is the meeting point of 2 flow branches and spot 3 which continues the flow from spot 2 (See Fig. 2).

3.3   Completed Test Results

Based on the results of the study in Table 3, positive results of E. coli at each point with sample concentrations of 0.1 ml, 1 ml and 10 ml were indicated by the color of the colony in the form of metallic green, so there was no need for TPC examination. Dark purple indicates Enterobacter sp [6]. Pink colonies indicate the presence of Klebsiella sp [7]. Klebsiella produces mucoid growth with a large polysaccharide capsule, while Enterobacter has a smaller capsule [8]. These three bacteria can be found because of the high activity of livestock that dispose of cow dung directly. to the river. Other causes may arise because of the possibility that people defecate directly into the river. Mammalian feces is the most common place for these three bacteria which are Escherichia coli, Klebsiella sp and also Enterobacter sp (see Fig. 3).

Table 3. Completed test results

Sample	0,1 ml	1 ml	10 ml
Spot 2 morning	Metallic green, Dark purple	Metallic green Pink	Pink, Dark purple
Spot 3 morning	Metallic green, Dark purple	Metallic green, Dark purple, Pink	Metallic green, Pink
Spot 2 daylight	Metallic green, Pink	Metallic green, Pink	Metallic green, Pink
Spot 3 daylight	Metallic green, Pink	Pink	Metallic green, Pink
Spot 2 Afternoon	Metallic green, Pink	Pink	Pink, Dark purple
Spot 3 afternoon	Metallic green, Pink	Pink	Pink

Fig. 3. Completed test results.

4 Conclusion

The total number of coliforms found in upstream water samples from the Cikapundung River came from two spots, namely at spot 2 which is located at the meeting point of the Angling drainage with the Cikapundung River and spot 3 which is located in the Cikapundung River stream behind The Lodge based on the Most Probable Number method of 1101 CFU/100 ml. Spot 1 which is located in Buleng as a water source or control has not been contaminated by coliforms. On the Completed Test examination, Escherichia coli was found to be a positive colony so that it did not meet the microbiological requirements. The results of the MPN test on water samples at Spot 2 and Spot 3

of the Cikapundung River did not meet the water quality standards for sanitation hygiene purposes based on the Regulation of the Minister of Health of the Republic of Indonesia No. 32 of 2017. Spot 1 which is located in Buleng as a spring or control still meets microbiological requirements. Researchers suggests several things that can be used as suggestions based on the research conducted, namely providing input to the Walungan Foundation and policy makers such as community leaders for follow-up so that education is carried out to the community and breeders not to pollute the Cikapundung River Area by not throwing waste directly into the river. Further research is needed to compare samples taken in other seasons such as the dry season and taken at other points such as near the PDAM inlet/outlet. Research can be used as a basis for community service or cross-sectoral collaboration with livestock or cross-programs with Citarum Harum.

References

1. D.T.B. Lestari dan H. Suprapto, "Analisis Pemanfaatan Mata Air Sebagai Sumber Air Baku", Jurnal Desain Konstruksi, vol. 16, no. (2), pp. 151-164, 2017.
2. Menteri Kesehatan Republik Indonesia. Peraturan Menteri Kesehatan Republik Indonesia Nomor 32 Tahun 2017 Tentang Standar Baku Mutu Kesehatan Lingkungan Dan Persyaratan Kesehatan Air Untuk Keperluan Higiene Sanitasi, Kolam Renang, Solus Per Aqua dan Pemandian Umum. Peratur Menteri Kesehat Republik Indonesia. Published online, 2017, pp. 1-20.
3. P. Wiliantari, I.N.K. Besung, dan P.G. K. Tono, "Bakteri Coliform dan Non Coliform yang Diisolasi dari Saluran Pernapasan Sapi Bali", Bul Vet Udayana, vol. 10, no. (1), p. 40, 2018.
4. R.S. Pakpahan, I. Picauly, dan I.N.W. Mahayasa, "Cemaran Mikroba Escherichia coli dan Total Bakteri Koliform pada Air Minum Isi Ulang", Kesmas Natl Public Heal J., vol. 9, no. (4), p. 300, 2015.
5. E. Afiatun, S. Wahyuni, dan S. Merinda, "Strategi Optimasi Pemanfaatan Sumber Air Bantar Awi Sungai Cikapundung Terhadap Instalasi Pengolahan Air Minum Dago Pakar", J Community Based Environ Eng Manag. vol. 2, no. (2), p. 51, 2019.
6. D. Darna, M. Turnip, dan R. Rahmawati. "Identifikasi Bakteri Anggota Enterobacteriaceae pada Makanan Tradisional Sotong Pangkong", J Labora Med., vol. 2, no. (2), pp. 6-12, 2018.
7. N.A. Ramaditya, P.G. K. Tono, I.G.K. Suarjana, dan I.N.K. Besung, "Isolasi Klebsiella Sp. Pada Sapi Bali Berdasarkan Tingkat Kedewasaan Dan Lokasi pemeliharaan Serta Pola Kepekaan Terhadap Antibakteri", Bul Vet Udayana, vol. 10, no. (1), p. 26, 2018.
8. R.N. Sunarti, "Uji kualitas air minum isi ulang disekitar kampus uin Raden Fatah Palembang", Bioilmi J Pendidik., vol. 2, no. (1), 2017.