

Qualitative Analysis of Coliform Bacteria in Hospital Wastewater with MPN Method

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Abstract- Objectives: Hospital wastewater is a waste product from hospital activities that are infectious and toxic so that it can affect health. In this case, the wastewater analyzed is wastewater in one of the Tasikmalaya City Hospitals. The purpose of this study is to determine the analyzed wastewater containing coliform bacteria that meets the requirements following Kep-58/MENLH/12/1995. The sample examination was carried out with the MPN method in the Microbiology Laboratory Bakti Tunas Husada Health Science College. The results of the analysis of coliform bacteria contamination of hospital wastewater using the MPN method containing coliform bacteria with an MPN value of 350 cells/100 mL. This shows that the content of coliform bacteria contained in hospital wastewater meets the requirements of hospital wastewater quality standards that are less than 1000 cells/100 mL. So the results obtained from these samples meet the requirements following Kep 58 /MENLH /12/1995.

Keywords: coliform, hospital, MPN, wastewater

I. INTRODUCTION

Water is an essential natural resource in life, water quality decreases because of contamination from bacteria and heavy metals caused by poor sewage systems and processes. Water contamination occurs when some materials or conditions that can cause a decrease in water quality so that it doesn't meet the quality standards or can't be used for certain purposes according to its purpose, such as hospitals.

One of the potential sources of water contamination is wastewater from hospitals. This is because hospital wastewater contains high levels of organic and inorganic compounds which also contain other chemical compounds and pathogenic microorganisms. All liquid effluents come from the results of the process of hospital activities that include liquid domestic waste and clinical liquid waste. Based on [1], hospital wastewater was found pathogenic microbes with the growth of microorganisms in water with variation turbidity levels range from 2 to more than 979/100 ml.

Coliform bacteria are a group of intestinal bacteria, an indicator of the presence of pathogenic bacteria and included in the group of microorganisms commonly used as indicators, where these bacteria can be a signal to determine whether a water source has been contaminated by a pathogen or not. These coliform bacteria produce ethionine which can cause cancer. Coliform bacteria can be used as an indicator because

the density is directly proportional to the level of water contamination.

The Most Probable Number (MPN) method was used to determine the number of coliform-type bacterial cells contained in hospital wastewater samples.

II. MATERIAL AND METHOD

A. Procedure

Wastewater Sampling

Sampling was carried out at 3 points of the collection with a certain distance from every sample. The sampling preparation was carried out aim to analysis the highest content of coliform bacterial contamination which will then be followed by qualitative testing.

Coliform bacteria contamination testing

a) Gram staining.

Aquadest dripped on glass objects are added 1 needle ose sample of wastewater, then fixation over fire. Drops the stain of violet crystal and leave for 1 minute, wash with water flow, then drop of Lugol leave for one minute and wash again with water flow. Then, drops 96% alcohol to leave for 10-20 seconds, wash with water flow and add safranin for 20-30 seconds then wash again with water flow, then dry using paper and add emersion oil and observe under a microscope.

b) Most Probable Number (MPN)

Media Preparation

Making Single and Double Lactose Broth Media

A total of 13 grams of Lactose Broth media was put into a beaker and dissolved in 1L aquadest. Then it is put into a magnetic heater while heated until dissolved, then taken 10 mL and put into 10 reaction tubes, every of which contains a Durham tube. For LB double, 52 grams of Lactose Broth media were weighed, then put into a beaker and dissolved in 1L aquadest. Then put into a magnetic stirrer until dissolved, then taken 5 mL and put into 5 reaction tubes, every of which contains a Durham tube. Then every media tube was sterilized in an oven with atm pressure at 121°C for 15 minutes.

Making Media Brilliant Green Lactose Broth

A total of 40 grams of Brilliant Green Lactose Borth media was put into a beaker and dissolved in 1 liter of distilled water. Then put into a magnetic stirrer while heated until dissolved,

then taken 10 ml put into a test tube containing a Durham tube. Then sterilized in an oven with atm pressure at 121°C for 15 minutes.

Presumptive Test

Arrange the 5 test tubes containing thick Lactose Broth media and 10 test tubes containing thin Lactose Broth media on the test tubes where every tube has been marked with a sample number and sample volume then shake until homogeneous. Insert 10 mL of sample into test tubes containing thick Lactose Broth media, and 1 mL every into 5 test tubes containing thin Lactose Broth media then the remaining 0.1 mL. Insert the entire tube into the incubator at 35°C for 2 x 24 hours. Then the formation of gas that occurs in the Durham tube is observed. The positive tube is then tested for affirmation.

Confirmatory Test

The tubes that were tested positive from the presumptive test were inoculated into tubes containing BGLB (Brilliant Green Lactose Broth) every one to two ose and carried out aseptic, then incubated at 35 ° C for 2 x 24 hours and after 48 hours of observation. The reading of the results is done by counting the number of positive tubes. The numbers obtained are adjusted with the MPN table [2].

III. RESULTS

Coliform bacteria contamination testing

Gram staining

The staining test was performed on three wastewater samples, the test results showed that in sample no 2 showed more dominant gram-negative bacteria so that the sample used in testing the MPN value was at sample point 2.

Most Probable Number (MPN)

Table 1: MPN presumptive test results from hospital wastewater sample

Positive tube			MPN/100 mL
5 Tubes of 10 mL	5 Tubes of 1 mL	5 Tubes of 0,1 mL	
5	4	5	430

The results obtained are compared with the MPN value. The results of this presumptive test were obtained MPN value of 430 cells/100 mL. Because the sample showed positive results, the test continued to the next stage, namely the affirmation test.

Table 2: MPN confirmatory test results from a hospital wastewater sample

Positive tube			MPN/100 mL
5 tubes of 10 mL	5 tubes of 1 ml	5 tubes of 0,1 mL	
5	4	4	350

The results of this affirmation test showed an MPN value of 350 cells/100 mL.

IV. DISCUSSION

Gram-negative bacteria is an indication of the presence of fecal and non-fecal coliform bacteria groups such as *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella* and *Klebsiella* which cause contamination so water quality decreased because it is pathogenic.

The tests include presumptive and confirmatory tests using the 5-5-5 tube series method. The results of this presumptive test were obtained MPN value of 430 cells/100 mL. Positive results are characterized by turbidity, presence of gas, and pH in Durham tubes. Because groups of coliform bacteria are aerobic and facultative anaerobic and don't form spores so that they can produce gas in the lactose broth media within 2x24 hours at a temperature of 35-40°C. This tube series test method is intended to fertilize the growth of coliform in order to obtain a value to estimate the number of coliform in the sample tested. The number of coliforms is not an exact calculation but is an actual number.

In this test, the sample used is the sample taken from a previous test from a tube that was tested positive by inoculating one to two ose into the BGLB (Brilliant Green Lactose Broth) media that had been equipped with Durham tubes. The Brilliant Green Lactose Broth media is used because it is a special medium for detecting gram-negative bacteria and as a selective medium for bacteria other than coliform. This media is able to inhibit the growth of gram-negative bacteria and increase the growth of coliform bacteria, to determine the presence of coliform bacteria (gram-negative) by the formation of gas and turbidity that occurs because to lactose fermentation by coliform group bacteria. This confirmatory test is done to confirm that the gas formed is derived from coliform bacteria not from other bacteria.

The results of this affirmation test showed an MPN value of 350 cells/100 mL. This shows that coliform bacteria contamination contained in hospital liquid wastewater still meets the hospital liquid waste quality standard requirements because the number of cells contained in hospital wastewater isn't more than 1000 cells/100 mL. Hospital wastewater quality parameters according to [3] that for the microbiological parameters of coliform bacteria the maximum amount is 1000 colonies/100 ml and doesn't exceed the threshold, if it exceeds the effect of hospital waste on environmental quality is very influential for health and can cause various problems. The positive impact if coliform bacteria can be used to help reduce heavy metal ion contamination from metal-contaminated wastewater through the mechanism of changing the chemical properties of the compound-forming structures as bioaccumulation, biotransformation, and bioremediation through the bioleaching process, but the number of bacteria doesn't exceed the maximum limit because if it exceeds the maximum limit because the boundary will cause disease for the surrounding environment.

Referring to [2] about the hospital's environmental health requirements, supervision of the existing waste management

system in the hospital is needed so that health services are more qualified along with the increasing need for health services.

V. CONCLUSION

Based on the research that has been done, it can be concluded that hospital liquid waste contains 350 cells/100 mL of coliform bacteria, this shows that the number of coliform bacteria is safe because it still meets the hospital quality wastewater quality standards that are less than 1000 cells / 100 ml. And it is necessary to do a reinforcement test on the analysis of the number of coliform bacteria using the media EMBA (Eosin Methylene Blue Agar) to determine the types of fecal coliform bacteria contained in the sample.

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