ORIGINAL ARTICLE

Determination of coliform bacteria sheet on water well in Bukittinggi City

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Abstract

Aim: The aim of the study was to know the quality of drill water which is close to the source of high pollution in Bukittinggi City that is suitable for public consumption. **Materials and Methods:** Using most probable number (MPN) and total plate count (TPC) method were used. **Results and Discussion:** The number of MPN >2400 bacteria/100 ml in sample A, 150 bacteria/100 ml in sample B, and >2400 bacteria/100 ml in sample C. As for TPC calculation result obtained 3752 x 10^2 *coliform*/ml in sample A, 4414 x 10^2 *coliform*/ml in sample B, and 5941 x 10^2 *coliform*/ml in sample C. **Conclusion:** Drilling water near the source of high pollution in Bukittinggi City is contaminated with *coliform* bacteria. Drilling water near the source of high pollution in Bukittinggi City is not feasible to be consumed when compared to good quality condition according to Ministry of Health Republic of Indonesia 2010 and Indonesian National Standard (SNI) number 01-3553-2006.

Key words: Coliform bacteria, contamination, the source of high pollution, well water drill

INTRODUCTION

All living things need water because water is one of basic need for life, especially humans, water is needed for various purposes, including household, industry, agriculture, and so forth.[1] Water is divided into two types, shallow well water and deep well water.[2] Shallow well water is water derived from a layer of water in shallow soil, typically ranging from 5 to 15 m from the soil surface, while deep well water comes from a second water layer in the soil. This kind of water was drilled from over 15 m from the soil surface.[3] Therefore, most of the deep well water is well enough to be used as drinking water, which directly without going through the processing.[4]

Drilling well water is deep well water which is drilled by inserting the pipe into it so as to get one layer of water. The presence of this water is caused by water absorption from the soil surface. This water depth ranges from 100 to 300 m from the ground.[5] If the pressure is high, then the water can gush out to the surface. This is called the artesian well. Meanwhile, if the water cannot get out by itself then the water will be gush out using the pipe.

In fulfilling the needs of water, people always pay attention to the quality and quantity of water. Sufficient quality is obtained easily because of the hydrological cycle, which is the scientific cycle that regulates and allows the availability of surface water and groundwater. However, population growth and human activities cause pollution which will make people difficult to obtain consumed water which meets the required standard.[6]

Improving the quality of drinking water by managing the quality of water is absolutely necessary, especially when the water comes from surface water. While increasing the quantity of water is the second condition after quality, because the more advanced one's life level, the higher the quality of water needs of the community.[7] For the drinking purposes, it takes average 5 l/day, while overall the need for water for an Indonesian household is estimated at 60 l/day. Therefore, the need for water for developed countries is certainly greater than the need for developing countries.

Coliform bacteria are microscopic organisms that originate in the intestinal tract of warm-blooded animals and are also present in soil and vegetation. Total coliform bacteria are generally harmless;

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however, their presence in drinking water indicates the possibility that disease-causing bacteria, viruses, or parasites (pathogens) are also present in the water.[8] Water is one of the carriers of disease that comes from feces to reach humans. To prevent water which enters the human body does not carry any disease, the water treatment either from source, transmission, or distribution network is absolutely necessary to prevent the occurrence of contact between impurities as a source of disease with water that is necessary.[9] Water that meets health requirements has an important role in maintaining, protecting, and enhancing public health status. On the basis of this understanding, below will describe some of the water quality requirements according to the World Health Organization (WHO), among others:

1. Physical terms, such as not colored, no smelly, not tasty, must be clear, and preferably at about 25°C(10).
2. Terms of chemistry
   Water should not contain toxins, mineral, or chemicals substance in quantities that exceed pre-determined limits.[10]
3. Bacteriological conditions
   Regulation of the Minister of Health of the Republic of Indonesia number: 492/Menkes/Per/IV/2010. Water should not contain bacterial diseases (pathogens) altogether and should not contain coli bacteria beyond the prescribed limits, that is, 0coli/100 ml water. Air containing coli groups is thought to have been contaminated with human waste.

The sources of water pollution include latrines, water disposal/comberan, landfills, and cattle pens. Technical guidance of water quality improvement for Environmental Health Development Officer,[11] the criterion in the provision of clean water, i.e., if the location of pollutant source is higher than the well, and it is estimated that the water will flow to the well the minimum distance of the well to the source is 11 m. However, if the location of the pollutant source is lower than the well, the minimum distance of the well to the source is at least 10 m (Ministry of Health RI, 1991).

Through the provision of clean water both in terms of quality and quantity in an area, then the spread of infectious diseases can be reduced to a minimum.[12] The decline of the disease is based on the consideration that water is one of the links to contagious diseases such as typhoid fever, dysentery, cholera, and infectious hepatitis.[13] For a person to remain healthy is strongly influenced by human contact with food and drink.[14]

The purpose of this research is to know the quality of drill well water which is located close to the source of high pollution that is suitable for public consumption.

MATERIALS AND METHODS

Tools

Autoclave, test tube, Durham tube, Erlenmeyer, becker glass, micropipette, Petri dish, colony counter, spirit light, ose needle, reaction tube shelf, cotton, gauze, paper parchment, matches, funnel, incubator, water bath incubator, stirrer bar, plastic sample, and icebox were used.

Materials

Well water drill near the source of high pollution in Bukittinggi City, lactose broth (LB), Brilliant Green Lactose Broth, Endo agar, Aquadest, and ethanol 70%.

Sterilization

Sterilization is the removal process of all life forms, either pathogenic, nonpathogenic, vegetative, or nonvegetative forms of an object or material.[15] The reaction tube, Durham tube, Petri dish, sample bottle, volume pipette, and becker glass are washed and dried first before use. The cotton sample bottle and the volume pipette are wrapped with parchment paper separately. Then sterilize by autoclaving for 15 min at 121°C.The ose needle is sterilized by means of a spray using a spirit light. Aseptic tables and cabinets are sterilized by cleaning them from dust and then sprayed with 70% ethanol.[16]

Testing Most Probable Number (MPN)

To test the water quality, we can use the MPN method,[17] which is also called the closest approximation by counting the number of bacteria present in the sample. An estimate test is used to look at the presence or absence of *coliform* bacteria based on the formation of acids and gases caused by lactose fermentation by coli bacteria.[18] The formation of acid is seen from the opacity of the lactose medium, and the resulting gas may be the air bubbles visible from the Durham tube.[19] An affirmation test is performed when positive results are obtained on an approximate test. If the result is positive, there is a gas bubble in the Durham tube incubated at 37°C for coliform bacteria, and incubated at 44°C for the fecal coliform bacteria. The reading is done after 48 h. Note the number of positive and negative tubes, then shake, see with table MPN/100 ml sample.

The method of examination of drinking water originating from the wellbore uses nine test tubes also called clean water checks.[2] This method is used for unprocessed samples, usually using: 3 ml × 10 ml, 3 ml × 1 ml, 3 ml × 0.1 ml.

Test forecast

a. Prepare nine test tubes for LB, three double-strength test tubes, and six single-strength reaction tubes, in which the Durham tube has been inserted.[20]
b. Divide the nine test tubes into three groups:
   1. Group 1, as many as three tubes (double strength).
   2. Group 2, as many as three tubes (single power).
   3. Group 3, as many as three tubes (single power).

c. Insert LB medium into three groups reaction tubes each containing 5 ml, for double strength.[21]
d. Then into three groups reaction tubes each containing 10 ml, for single strength.
e. And into three tubes of reaction Group 3 each containing 10 ml, for single strength.
f. Insert water sample into three groups reaction tubes 1 each 10 ml, shake homogeneously.
g. Into the Group 2 reaction tubes each 1 ml of homogeneous shake.
h. And into three groups reaction tubes each of 0.1 ml, shake homogeneously.
i. Close all test tubes with cotton.
j. Incubate at 37 ° C for 48 h.
k. Observe each test tube to see is there any gas is in the Durham tube. If there is any gas in Durham tube, estimates showed a positive test, but not yet certain of the bacterium golongan coliform. Therefore, it is followed by an affirmation test.[21]

Test of affirmation

The affirmation test performed there are two, namely, the confirmation test for coliform and the confirmation test for the fecal coli.[22]

a. Take with the needle ose of each tube on a positive forecast test, transfer it to the Brilliant Green Lactose Bile Broth (BGLB) reaction tube.
b. Incubate at 37°C to confirm coliform and 44° bacteria for fecal coliform bacteria.
c. The reading is done after 48 h by looking at the number of positive gas BGLB tubes.
d. Record the number of tubes in the BGLB assay test that indicates both positive and negative gas. The number obtained is matched with the MPN table per 100 ml sample, it will get index MPN coliform bacteria incubated at 44°C.[23]

Total Plate Count Test (TPC)

To count the number of colonies in the sample can be done by TPC method.[24] The media which were used in this method are endo agar in a Petri dish. The calculation was performed after mixed dilution of the sample medium and incubated at 37°C for 24 h in reverse position. The total number of bacteria in 1 ml of sample is by multiplying the average number of colonies in the Petri dish by the dilution factor of the samples present.[25]

a. Samples from each well bore water were taken 500 ml through the tap and put into aseptic sterile bottles.
b. Provide 15 dilution squash, and 30 Petri dish.
c. For the first dilution, put 1 ml sample into a sterile flask A which has contained 9 ml of Aquadest and shake it homogeneously. For the second dilution, put 1ml first dilution's result into sterile flask B which has nine Aquadest and shake it. For the third dilutions, put 1 ml of second dilutions result into sterile flask C which has contained 9 ml Aquadest and shake it. The same treatment is performed until the fifth dilution.
d. In the second and third sample bottles do like the above method.
e. From each dilution result, put 1 ml into the Petri steril plate, then poured 15 ml sterilized endo agar, then the cake was immediately shaken and rotated until the medium and the sample homogeneous.
f. After the media solidifies, incubate the Petri dish at 37°C for 24 h in an upside position.
g. Count the growing colonies on each Petri dish.
h. The total number of bacteria in 1 ml of the sample is by multiplying the average of colonies in Petri dishes by dilution factor.[26]

RESULTS

MPN

From research which has been conducted on the MPN coliform and fecal coli, then obtained the data as follows:

a. The research result of the estimated test of MPN
b. Test of affirmation.

TPC

Using the TPC formula, the following results are obtained:

a. In Sample A the number of colonies: 3752×10^2 coliform/mL sample
b. In Sample B the number of colonies: 4414×10^2 coliform/mL sample
c. In Sample C the number of colonies: 5941×10^2 coliform/mL sample.

DISCUSSION

In this research, the testing of MPN and TPC number on three water samples is taken from drilled well near to source of high pollution in Bukittinggi City. In the MPN test, double tube method using nine test tubes. According to the Ministry of Health of Indonesia (1995), this method is a common method used to test the content of coliform bacteria in unprocessed samples, such as well water, river water, and others.[27] The MPN method consists of two types of tests, namely, test estimates and assertion tests. The forecast test aims to determine whether or not the coliform bacteria are present in the sample while the assertion test aims to ensure that the bacteria contained in the sample are a coliform or fecal coliform bacteria.[28] Coliform bacteria derived from plants or animals that have died, for example, *Enterobacter aerogenes,*[29] while fecal coliform bacteria derived from human and animal feces, for example, *Escherichia coli*.[30]

The medium used in the approximate test is the LB with different amounts and strengths according to the number of samples added. Nine test tubes are divided into three groups, each of three test tubes. The first three test tubes

were filled with 5 ml of LB medium with double strength, while the other test tubes filled 10 ml of medium with a single power. Samples and media are inserted into a test tube containing the Durham tube in reverse position. Durham tube serves to provide air cavity as a result of bacterial lactose fermentation.[31]

The entire test tube contains sterilized media before adding the sample, using an autoclave with a temperature of 121°C for 15 min.[32] After sterile, each medium is filled with samples. Then, the entire test tube is closed using cotton swabbed cloth. All the work is done inside the aseptic cabinet. The goal is that the supply of air, materials, equipment, and personnel can be controlled in such a way that microbial contamination remains at an acceptable level.[32] The reaction tube contains the media, and the sample is incubated with 37°C. Calculations were performed after bacteria were cultured for 48 h [Table 1].

Positive results are characterized by the appearance of gas bubbles in the Durham tube. If the forecast test shows a positive result, then proceed with an affirmation test. This test is performed by inoculating positive bacterial cultures of positive test results into the BGLB medium inside the test tube containing the Durham tube in reversed position. BGLB media are a selective medium that can inhibit the growth of Gram-positive bacteria that cannot live in the digestive tract. In addition, BGLB also contains a brilliant green that can inhibit the growth of Gram-negative bacteria other than *E. coli*. Bacterial inoculation was performed using a sterile syringe needle inside the aseptic cabinet into two test tubes of each test tube bubbling on the approximate test. Once inoculated, close the test tube using a cotton swab. The reaction tube containing the BGLB media and bacterial culture was then fed into the incubator at 37°C and water bath incubator at 44°C.

Calculations were performed after bacteria were cultured for 48 h. Coliform group bacteria showed positive results by generating bubbles of gas in Durham tube at 37°C,[33] while the fecal coliform bacteria would produce gas bubbles at 44°C[30] [Table 2]. The sources of water pollution include latrines, sewage, landfills, and livestock pens.[34] The criterion in the provision of clean water, i.e., if the location of pollutant source is higher than the well and it is estimated that the water will flow to the well the minimum distance of the well to the source is 11 m. However, if the location of the pollutant source is lower than the well, the minimum distance of the well to the source is at least 10 m[35] (Ministry of Health RI, 1991).

TPC is a method used to calculate the number of bacterial colonies.[36] In this method, media used are endo agar media because according to Acumedia Manufacturers, this medium is specific to count coliform bacterial colony.[37] With dilution, prepared five pieces of sterile reaction tube containing 9 ml Aquadest. Each of the tubes added 1 ml sample to be examined gradually, that is 1 ml sample into the first tube, until the concentration of the solution in the first tube becomes 10^{-1}. 1 ml from the first tube is inserted into the second tube until the concentration of the solution becomes 10^{-2} and so on until it reaches the solution with the lowest concentration. The dilution results are fed into sterile Petri dishes of 1 ml using sterile syringes. Then, 15 ml of the endo agar medium was sterilized, homogenized, and waited until solid. The work is done inside the aseptic cabinet. The densely packed media is incubated at 37°C in an upside position to prevent water vapor from dripping onto the surface. This method is done Duplo to get more accurate results. The calculation is done after 48 h using colony counter. The calculated cup is a cup whose colony counts between 30 and 300. Grapes with colonies of less than 30 and more than 300 cannot be used because they are statistically unreliable.

Results obtained from this study indicate that the TPC of Sample A is 3752 × 10^2 *coliform*/mL sample, B 4414 × 10^2 *coliform*/mL sample, and C 5941 × 10^2 *coliform*/mL sample [Table 3]. The third does not meet the requirements set by the National Standards Board, which is 1.0 × 102 colonies/mL [Table 4].

Table 1: Estimated test of most probable number

Sample	Volume			Most probable number/100 ml
	10	1	0,1	
A	3	3	3	>2400
B	3	2	1	150
C	3	3	3	>2400

Table 2: Test of affirmation number most probable number

Temperature	Sample	Sample volume (ml)		
		10 ml	1 ml	0,1 ml
37°C	A	+++	+++	+++
	B	+++	++	+
	C	+++	+++	+++
44°C	A	+++	+++	+++
	B	+++	++	+
	C	+++	+++	+++

Description: + is a reaction tube that produces bubbles

Table 3: Number of bacterial colonies found in Petri dishes

Dilution	Sample A		Sample B		Sample C	
	C_1	C_2	C_1	C_2	C_1	C_2
10^{-1}	548	580	470	504	562	614
10^{-2}	212	238	220	287	297	294
10^{-3}	120	130	183	176	225	204
10^{-4}	59	74	53	61	103	120
10^{-5}	24	21	20	31	42	34

Table 4: Boundary of microbial contamination in drinking water according to SNI 01-3553-2006

Test criteria	Unit of measurement	Requirement	
		Mineral water	Demineral water
Total plate number	*Coloni*/ml	Maximum 1.0×10^2	Maximum 1.0×10^2
Cole-shaped bacteria	APM/100ml	<2	<2
Salmonella	-	Negative/100 ml	Negative/100 ml
Pseudomonas aeruginosa	*Coloni*/ml	Nol	Nol

(SNI 01-3553-2006)

Methods were used in this study equal to previous studies, where household ice cubes in the Aur market of Bukittinggi are not feasible for consumption when compared to the maximum bacterial contamination limits set by the Indonesian National Standardization Agency use TPC methods.[38] Another research about water is coliform contamination in swimming pool water. Water recreation, though increasing globally, is strongly associated with infectious diseases. Ten unexpectedly, artificial water recreation systems, for example, swimming pools account for 90% of these 11 outbreaks. It is, therefore, essential that pool waters be regularly monitored for deviations from 12 microbial water quality guidelines. The result is coliform counts and detection of *E. coli* clearly violates 20 international guidelines. Pool operators should increase water disinfection efficiency 21 and educate the public on the need for improved swimmer hygiene to reduce the risk of recreational 22 water illness transmission.

Another research about microbiological (coliform) in drinking water source has done in Kalar City, from the different drinking water samples shows the water from (dug well water) are not suitable for drinking according to WHO standars, but drilled water is better for drinking and the normal tap water is much better for drinking beacuse it has been filtrated by government.[8] This is same with drinking water resource research in Pakistan, where in Pakistan there is not a good awareness about water-borne diseases. It is just due to lack of knowledge and infrastructure, and it is not a hidden thing that in Pakistan water borne diseases are not different from world, so that disinfection of water should be implemented to reduce water-borne diseases, water supplying departments have to follow WHO standards for better public health and to control disease outbreak by coliforms.[39]

The routine examination of the drinking water filtration system always related to the monitoring the levels of turbidity, determination of the relative clarity of water, and presence of microorganisms. Turbidity is reasons through matter, for example, clay, silt, fine organic and inorganic matter, plankton, and other microscopic organisms, which could be suspended within the water. Three kinds of microorganisms that could be found in drinking water are bacteria, viruses, and protozoa.[39]

Bacteria coliform found in environment and feces from warm-blooded animals and also found in humans, this bacteria normally cause disease and found a lot in water, most dangerous water is contaminated bacteria coliform derive from animals dan humans, test in water that retains pathogenically should do. If that water is used to drink has contaminated the coliform bacteria, so humans should keep much attention to the source of water and heat water correctly. When coliform bacteria are found, water systems investigate to find out how the contamination got into the water. They collect additional water samples and often inspect the entire system. Collecting additional samples helps determine whether an actual problem exists. If the lab detects bacteria in any of the additional samples, the initial findings are "confirmed."

CONCLUSION

From research determination of coliform bacteria contamination on drilled water well in Bukittinggi City can be concluded:

1. Drilling well water near the source of high pollution in Bukittinggi City is contaminated with *coliform* bacteria.
2. MPN of well bore water close to high pollution in Bukittinggi City exceeds good water quality standard according to Ministry of Health Republic of Indonesia year 2010.
3. TPC in drilled well water taken in areas close to the source of high pollution in Bukittinggi City have TPC numbers exceeding good water quality standard according to Indonesian National Standard number (SNI) 01-3553-2006.
4. Drilling well water near the source of high pollution in Bukittinggi City is not feasible to be consumed when compared to good quality condition according to Ministry of Health Republic of Indonesia 2010 and Indonesian National Standard Number (SNI) 01-3553-2006.

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