

## Isolation and identification of escherichia coli in well water located in Garut Regency

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### ABSTRACT

Water is one of the most important needs for daily life, especially well water. In the Garut district area well water is used for daily activities ranging from washing, drinking, bathing, ablution to being used for drinking water consumption. This study aims to isolate and identify well water from 10 villages located in the Garut Regency. Sampling was done using purposive sampling method. The test was followed by a presumptive test using Lactose Broth (LB), a reinforcing test using 2% BGLB media and a complementary test consisting of EMB agar media and biochemical tests. The results showed that of the 10 well water samples, 7 met the requirements of proper water quality and 3 did not meet the requirements of clean water with MPN values from three villages, namely Kampung Tanjung kamuning 200/ml, Kampung salaawi 241/100ml and Kampung Cipepe 241/100ml. EMB agar media is characterized by the formation of a metallic green color with black spots colonies and biochemical tests that show the well water has been contaminated with *Escherichia coli* bacteria.

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## INTRODUCTION

Water is one of the most important materials needed by humans. Humans are the living creatures that are most easily infected with diseases through water (Jasper, 2020)(Baroni et al., 2008). People consume water from various sources such as groundwater, well water and also refill water. Clean water is the safest water for consumption and must also meet health requirements to be consumed. It will be safe if the water meets the health requirements of physical, microbiological, chemical, and radioactive requirements. Nowadays, along with the development of population growth, the availability of clean water is reduced. The level of water pollution is one of the main problems that must now be faced (Soltani et al., 2007). What must be seen from the quality of water is from the type of soil in the Garut Regency which includes alluvial soil types, Andosol associations, Lithosol associations, Mediteran associations, Podzolic associations, and Also regosol associations. These soil types each have certain properties that can be a potential or constraint in certain land uses (Huang, 2021)

Water is the most important need for the human body because humans cannot survive without water, therefore most of the human body about 70% contains water for fluid balance in the

body, especially to prevent dehydration. Some of the benefits of water needed by humans include to sustain life starting from primary needs for the body and secondary for the continuity of activities needed (Doherty & Poulin, 2022; Jamilatun & Aminah, 2016). From the primary benefits of water needed for the human body to avoid dehydration, to maintain skin moisture, and protect nerves and tissues for the body. The secondary needs of the benefits of water are used for washing, bathing, cooking, even needed for industry, firefighting, agriculture, and others (Agustina, 2021; Anyolitho et al., 2022).

The increase in population every year shows the increasing conditions that affect the community both in social and economic conditions, it will also increase the needs of the community in using water as a necessity of life, dug wells become a source of clean water supply for some communities where the origin of this water comes from the soil layer relatively close to the ground surface. Because this water is produced from layers of soil that are relatively close to the ground surface, it is easily contaminated with human and animal feces, as well as household cosmetic waste. If the dug well water is identified with *Escherichia coli* bacteria, it is a sign that the water has been polluted by human feces, and currently in urban areas it has reached 70% of water infected with human feces (Griebel et al., 2022; Hajare et al., 2022).

According to Enjang (2000), the distance between wells and latrines, disposal pits, waste pits and other sources of contamination should not be less than 10 m because it can cause contamination of well water by bacteria. Meanwhile, according to Chandra (2007) the distance of the well is at least 15 m and is located higher than sources of pollution such as latrines, livestock pens, garbage bins and so on. In addition, to prevent seepage or return of water into the well, the well must have walls made of brick or cement and a floor made of cement. According to PERMENKES RI No.416/MENKES/PER/IX/1990 on the requirements and quality of clean water which stipulates the amount of *Escherichia coli* in clean water is 0/100 ml samples of *Escherichia coli* bacteria were chosen as sanitation indicators because *Escherichia coli* bacteria are normal flora that exist in the intestines of humans and the intestines of warm-blooded animals (Haribi & Yusron, 2010; Pratama et al., 2020).

## RESEARCH METHOD

This study uses a laboratory descriptive method, the subject in this study used 10 well water samples located in various areas of the city of Garut namely Kampung Cisanca, Kampung Genteng, Kampung Tanjung Kamuning, Kampung Salaawi, Kampung Cipepe, Kampung Excello, Kampung Munjul Kulon, Kampung Palnunjuk, Kampung Sukasirna, and Kampung Nusajaya. This method is carried out by looking at the development of bacteria in the observation of *Escherichia coli* analysis on the media, *Escherichia coli* bacterial analysis testing is carried out from the presumptive test, complementary test, and biochemical test.

### Sampling

Sampling was carried out using purposive sampling method. The sampling technique was carried out by water sampling, where in each well 1 bottle was taken in a volume of 600 ml of water from different locations so that the total number of tests was 10 samples.

### Calculation of Total Coliform Bacteria

#### Presumptive Test

At this stage the samples were planted in 3 series of tubes containing 10 ml Lactose Broth medium. A sample of 10 ml was planted in the first tube series, 1 ml in the second tube series, 0.1 ml in the third tube series. The tubes were incubated at 37°C for 2x24 hours. The tubes whose media changed color and produced gas continued with the confirmation test.

#### Confirmed Test

At this stage the tubes that were positive in the presumptive test were taken a little by dipping the ose into the tube and then inoculated into the Brilliant Green Lactose Broth (BGLB) tube 0.2%. After that it was incubated at 37°C for 2x24 hours. The tubes that produced gas were recorded and matched with the MPN table to determine the number of *Escherichia coli* bacteria contained in the sample.

### Completed Test

At this stage the positive Lactose Broth tube is characterized by a change in color and produces gas, inoculated with an ose into *Eosin Methylene Blue Agar* (EMBA) media. After that it is incubated in an incubator at 37°C for 1x24 hours. The presence of *Escherichia coli* is indicated by the formation of shiny bacterial colonies (metallic greenish red). Colony results were then inoculated into all biochemical test media consisting of *Simmon Citrate*, Methyl Red (MR) and carbohydrate fermentation (sugar) tests. The tubes were incubated in an incubator at 37°C for 1x24 hours. Positive *Escherichia coli* if the biochemical test results obtained *simmon citrate* (-), Methyl Red (+), Glucose (+), sucrose (+), lactose (+), Maltose (+) and mannose (+).

### Data Analysis

The research data is presented descriptively in the form of tables or figures. *Escherichia coli* observation data were analyzed using the Most Probable Number (MPN) method of 3-3-3 variety. *Escherichia coli* identification data are presented in the form of images.

## RESULTS AND DISCUSSIONS

In this study, the samples used were sourced from 10 wells located in several villages in the Garut district starting from Kampung Cisanca, Kampung Genteng, Kampung Tanjung Kamuning, Kampung Salaawi, Kampung Cipepe, Kampung Excelo, Kampung Munjul Kulon, Kampung Palnunjuk, Kampung Sukasirna and Kampung Nusajaya. Well water samples were then taken aseptically using contamination-free tools and materials. Testing well water samples using the MPN method is the first stage of conducting a presumptive test using Lactose Broth (LB) media. LB media is used as a medium to detect the presence of *Escherichia coli*, the way LB media detects *Escherichia coli* is based on the formation of acids and gases caused by the fermentation of lactose by *Escherichia coli* bacteria. (Siti Nurkomala Sari, Ety Apriliana, Susianti, 2019). Interpretation of the results of this presumptive test is marked by a change in the color of the media which initially turns light pink to yellow and the formation of gas in the Durham tube. Tubes that show positive results are continued with an affirmation test using 2% Brilliant Green Lactose Bile Broth (BGLB) media. BGLB media is a medium used to detect the presence of *Escherichia coli* or gram-negative, interpretation of the results of BGLB media is characterized by the formation of gas bubbles in the Durham tube and the color of the media becomes cloudy, the positive results of the Affirmation Test are matched with the MPN *Escherichia coli* table (Dila Putri et al., 2022). The test results can be seen in Table 1 below:

**Table 1.** Results of observation of positive tubes in well water from presumptive tests on LBDS (Lactose Broth Double Strength), LBSS (Lactose Broth Single Strength) and MPN tests using BGLB (Brilliant Green Lactose Broth) 50/100 ml media.

No	Sample	LBDS (10ml)	LBSS (1ml)	LBSS (0,1ml)	Positive Tube Combination	MPN <i>Escherichia coli</i> Per 100 ml	Description
1	Kampung Cisanca	---	---	---	0-0-0	0	Eligible
2	Kampung Genteng	+ - +	+ - +	---	2-2-0	20	Eligible

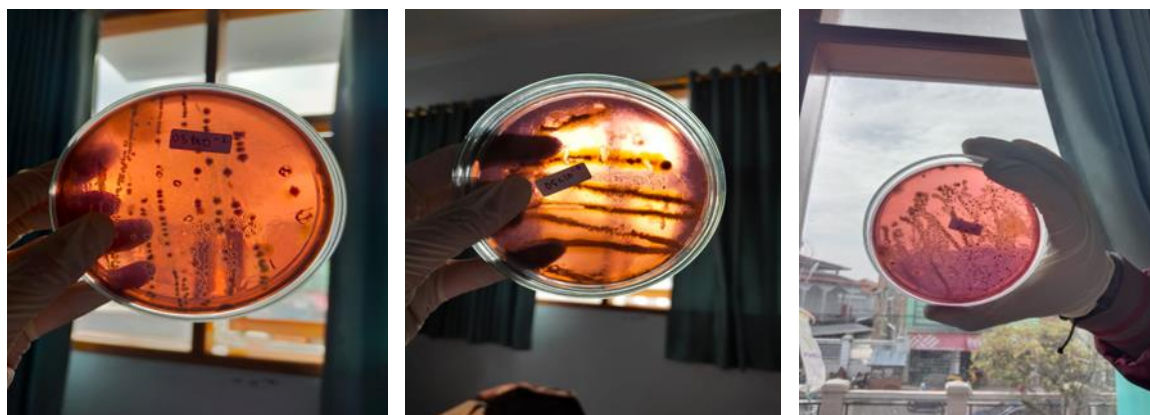
3	Kampung Tanjung Kamuning	+++	+ - +	- - +	3-2-1	200	Not Eligible
4	Kampung Salaawi	+++	+++	---	3-3-0	241	Not Eligible
5	Kampung Cipepe	+++	---	+++	3-0-3	241	Not Eligible
6	Kampung Excelo	+++	---	---	3-0-0	21	Eligible
7	Kampung Munjul Kulon	+++	---	---	3-0-0	21	Eligible
8	Kampung Palnunjuk	---	- - +	+++	0-1-3	48	Eligible
9	Kampung Sukasirna	++-	---	- + +	2-0-2	21	Eligible
10	Kampung Nusajaya	++-	---	---	3-0-0	25	Eligible

Description: (-) Not growing

(+) Growth Forms Gas and Media becomes Cloudy

Based on the test results in Table 1. that has been done, of the 10 well water samples located in the Garut district there are 7 well water samples that meet the requirements and 3 well water samples do not meet the requirements. 3 samples of unqualified well water are from Kampung Tanjung Kamuning 200/100 ml, Kampung Salaawi 241/100 ml and Kampung Cipepe 241/100 ml. Well water samples taken are well water used for daily activities, one of which is for consumption as drinking water. According to PERMENKES 492/MENKES/PER/1V/2010 regarding water quality requirements that are suitable for public consumption do not cause health problems. These requirements include chemical, physical and bacteriological requirements, where for the bacteriological MPN (Most Probability Number) value is 0/100 ml (PERMENKES, 2010). MPN values that exceed the standard indicate that the well water has been contaminated with *Escherichia coli* bacteria. Well water that has been contaminated with *Escherichia coli* bacteria and is not clean can have a negative impact on the health of people who use the water for drinking, because the content of microbes if it exceeds the standard standard can cause diarrhea. The discovery of *Escherichia coli* bacteria in well water indicates that the well water has been contaminated with human and animal feces and contains intestinal pathogens, which can cause food poisoning if ingested along with food or drinks to be consumed (Agustina, 2021).

Furthermore, a complementary test is carried out if the reinforcing test, namely 2% BGLB media, is positive or does not meet the quality requirements of good drinking water. Well water samples from 3 villages from Kampung Tanjung Kamuning, Kampung Salaawi and Kampung Cipepe. tested positive so that the samples were cultured on Eosin Methylen Blue agar (EMBA) media. Interpretation of the EMBA test the formation of metallic green colonies on the media is shown in Figure 2.



**Figure 1.** Complementary test results on EMB agar media for the three well water samples that tested positive

The identification results of well water samples on EMB agar media in Figure 3 show metallic green colonies with an approximate diameter of 2-3 mm with a black dot in the center of the colony. This color change occurs because *Escherichia coli* ferments lactose so that there is an increase in acid levels in the media. High acid levels can precipitate methylene blue in EMB agar media. EMB agar is known to be a selective media and differential media functioning to inhibit gram-positive bacteria and grow gram-negative bacteria (Jamilatun & Aminah, 2016). *Escherichia coli* is a gram-negative bacterium that is normal flora in the human digestive system and can also be a pathogen that causes infection. is known to be an enterotoxins cause of diarrhea, which is a health problem that is very often experienced by the community, especially in children in developing countries such as Indonesia, which is caused by environmental factors and behavioral factors (Pratama et al., 2020).

Samples that tested positive on EMB media were subjected to further biochemical tests. Biochemical test is a complementary test to determine the specifications of *Escherichia coli* bacteria. interpretation of the results of biochemical tests carried out obtained Simmon Citrate (SC) (-), MR (+), VP (-), Glucose (+), Sucrose (+), Lactose (+), Maltose (+) and Mannose (+). The results of the biochemical test can be seen in Table 2 below.

**Table 2.** Biochemical test results on *escherichia coli* bacteria

No	Sample	SC	MR	VP	Glukosa	Sukrosa	Laktosa	Maltosa	Manosa
1	Kampung Tanjung Kamuning	-	+	-	+ Gas	+	+	+	+
2	Kampung Salaawi	-	+	-	+ Gas	+	+	+	+
3	Kampung Cipepe	-	+	-	+ Gas	+	+	+	+

From the results of the research on biochemical tests in Table 2. It was found that the Simmon Citrate (SC) test was negative for *Escherichia coli* indicated by the absence of media changes in the SC test. SC is known to detect the ability of an organism to utilize energy and carbon sources. If the organism is able to use SC as its carbon source, it will raise the PH so that it changes the color of the media from green to blue. *Escherichia coli* is one of the bacteria that does not use Citrate as a carbon source in its environment. in the MR test the results obtained are positive marked by a red solution. MR test aims to detect the ability of organisms to produce and maintain stable end products from glucose fermentation. In the VP test the results obtained are positive, the red color indicates a positive result while the yellow-brown color indicates a negative result. The VP test will be negative for *Escherichia coli* bacteria because these bacteria are able to ferment carbohydrates into acidic products and do not produce neutral products. Meanwhile, the sugar test on *Escherichia coli* bacteria can ferment glucose, sucrose, lactose, maltose and Mannose characterized by positive results (Afrianti Rahayu & Muhammad Hidayat Gumilar, 2017).

Based on the results of testing starting from the presumptive test, booster test and booster test of 10 well water samples from several villages in the Garut district, there are 3 well water samples that are identified as contaminated with *Escherichia coli* bacteria. *Escherichia coli* is one of the microorganisms most often used as an indicator for determining or detecting pathogens in water because this bacterium has good resistance, is easy to isolate and easy to grow. So this bacterium is known to be a signal to determine whether a water source has been contaminated by pathogenic bacteria or not. One of the factors that can affect the presence of *Escherichia coli* bacteria is the depth of the water source, the distance between latrines and clean water sources, and the distance between septic tanks and water sources (Haribi & Yusron, 2010).

## CONCLUSION

The results of this study can be concluded that there are 3 *Escherichia coli* bacterial contamination in 10 well water samples located in the Garut district, namely in Kampung Tanjung Kamuning, Kampung Salaawi and Kampung Cipepe with MPN values of 200/100 ml, 241/100 ml and 241/100 ml respectively. In the reinforcement test using EMB agar media, positive results were found characterized by metallic green colonies with an approximate diameter of 2-3 mm with a black dot in the center of the colony on EMB agar media. Then, in the complementary test consisting of biochemical tests, the results indicate that the well water sample is not suitable for use for daily activities by the people of the Garut district.

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