ORIGINAL ARTICLE

Prevalence of Enteropathogenic Bacteria in Groundwater: Public Health Implications of Drinking Water Contamination in Jember, Indonesia

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ABSTRACT

Introduction: Groundwater is the most widely used source of potable water worldwide. However, the presence of enteropathogenic bacteria in drinking-water systems poses a growing public health concern, particularly in developing regions. This study aimed to assess the bacteriological quality of domestic groundwater in Jember, East Java, and to identify the presence of waterborne pathogens associated with gastrointestinal infection. **Methods:** A total of 42 groundwater samples were collected from household dug wells and analysed for total coliform counts. The presence of *Escherichia coli* and *Salmonella sp.* was confirmed using selective culture media and standard microbiological procedures in accordance with WHO drinking-water quality guidelines. **Results:** Only 3 of the 42 samples (7.1%) complied with the WHO standard of zero coliforms per 100 mL. In contrast, 29 samples (69%) were contaminated with enteropathogenic bacteria, predominantly *E. coli* and *Salmonella sp.*, indicating faecal pollution and a potential risk of waterborne disease transmission. **Conclusion:** These findings highlight the need for improved household water treatment, enhanced sanitation practices, and routine microbiological monitoring. Strengthening public health interventions is essential to ensure safe groundwater consumption and to reduce the risk of gastrointestinal infections in the Jember community.
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INTRODUCTION

The most important natural resource for human existence is water. Nothing on Earth could thrive without it. The amount of water that can be carried around in portable forms is found in groundwater, springs, rivers, and lakes, which make up only roughly 3% of the total (1). Increased population growth will lead to increased demand for clean and safe water. Humans rely heavily on groundwater to fulfill daily needs, which is expected to increase by about 1% per year for the next 30 years (2). The situation is even more significant in Asia and Africa, where groundwater is the main source of drinking water (3). Groundwater contamination is closely related to human activities, such as household activities, industry, market, etc. The quality of groundwater is primarily endangered by human actions, though natural processes can occasionally introduce detrimental substances (4). Accurately assessing the microbiological safety of water resources is critical to reducing waterborne diseases.

Some of the most reported bacteria in water sources are *Escherichia coli, Salmonella spp., Shigella spp.*, and *Staphylococcus aureus*. These bacteria have been linked to waterborne disease outbreaks (5). For gathering water from the head, transitional, and meandering portions of the Kelani River Basin for a full year (during both the dry and wet seasons), 72 groundwater and 45 surface water sampling points were chosen (6). The emergence of antibiotic-resistant microorganisms/antimicrobial resistance (AMR) in water sources has raised concerns recently (7). Due to this issue, it is important to monitor and investigate the microbial quality of water to effectively manage water quality and promote public health.

Groundwater is utilized for domestic, agricultural, and industrial purposes and is one of the world's primary sources of freshwater. Groundwater provides drinking water to almost one-third of the world's population (8). Bacteria that have developed antibiotic resistance are able to withstand and evade the effects of antibacterial drugs that were once effective in treating them. Regular discharge of antibiotics into natural aquatic habitats may lead to bacterial populations under selective pressure to develop antibiotic resistance to survive (9).

Jember Regency, East Java, Indonesia is characterized by widespread use of household groundwater wells and variable sanitation infrastructure. Despite the high reliance on groundwater, routine bacteriological surveillance of domestic wells remains limited. Data on the prevalence of enteropathogenic bacteria in household groundwater are essential to inform risk assessment and preventive strategies from a medical microbiology standpoint. Therefore, this study aimed to evaluate the bacteriological quality of household groundwater and to identify the presence of clinically relevant enteropathogenic bacteria associated with waterborne infections. The findings are expected to provide microbiological evidence to support targeted public health interventions and improve drinking water safety.

MATERIALS AND METHODS

Materials

The tools used in this study include glass bottles, Erlenmeyer glasses, beaker glass, ose, analytical scales, micropipettes, autoclaves, laminar airflow, petri dishes, sterile test tubes, incubators, and electric stoves. While the materials needed are MacConkey Broth media, Salmonella Chromogenic Agar (SCA) media, aluminum foil, cotton, gloves, ice gel, and syringe.

Sample Collection

This research took place from August 2023 - December 2023 conducted in 12 sub-districts in Jember Regency, including Sumbersari District, Pakusari District, Kaliwates District, Tanggul District, Balung District, Mayang District, Ambulu District, Silo District, Bangsalsari District, Sukowono District, Rambipuji District, and Kalisat District. These sub-districts were chosen because they have all 4 contaminant points to be studied, namely slaughterhouses, cemeteries, farms, and landfills. The samples in this study were 72 samples obtained from 6 samples/sub-districts near slaughterhouses, cemeteries, farms, and landfills. Water samples were collected as much as 100 mL and stored in sterile glass bottles that had been labeled. Samples that have been taken are then placed on Styrofoam that has contained ice gel to prevent bacteria from multiplying. The samples were then immediately brought back to the Microbiology Laboratory of the Faculty of Medicine, University of Jember for research and testing.

Bacterial Analysis

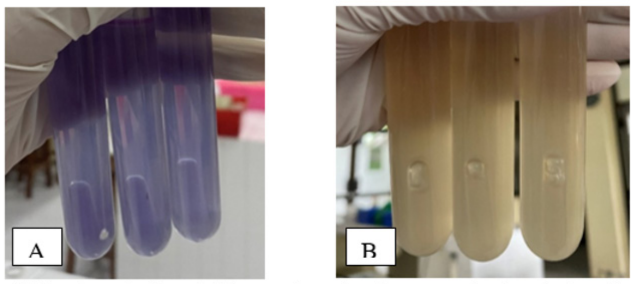
Samples were tested as soon as possible after arriving at the Microbiology Laboratory of the Faculty of Medicine, University of Jember. The tests carried out in this study were the Most Probable Number (MPN) test and bacterial identification using SCA media. MPN test using the 3x3 tube method with MacConkey Broth media. Samples of 10 mL, 1 mL, and 0.1 mL were then put into 9 test tubes containing MacConkey Broth solution. This test was conducted to determine the number of coliform concentrations in each well water sample. The MPN tube is said to be positive if bubbles appear in 10% of the tube on the Durham tube and the color changes from purple to cloudy in the MacConkey Broth solution. Meanwhile, bacterial isolation using SCA media is carried out by striking techniques to find out directly what bacteria the sample contains. SCA media can identify *Escherichia coli* and *Salmonella sp.* directly in one agar plate. The appearance of blue-green colonies on SCA media can confirm that the well water sample contains *E. coli*. While the appearance of pink colonies on SCA media indicates that the sample is positive for *Salmonella sp.* All these tests were carried out in laminar flow to prevent other contamination during the process. Furthermore, all samples that have been planted on all media will be incubated at 37ºC for 24 hours.

RESULTS

Identification of Bacteriological Quality of Dug Well Water

The bacteriological quality of dug well water in this study was examined using the MPN method and SCA media. The MPN value in the dug well water samples was determined by observing the formation of gas bubbles as much as 10% of the tube size in Durham tubes and the color change from purple to cloudy in the MacConkey Broth solution. The results of observations of the quality of bacteriological dug well water using the MPN method can be seen in Figure 1.

The MPN method is used to determine the concentration of coliform bacteria in dug well water samples. Table I shows the bacteriological quality of dug well water examined using the MPN method. Interpretation of coliform bacteria concentration using the Thomas Formula with units of MPN/100 ml. The observation data shows that there are 3 samples of dug well water (7.1%) that still meet the standard of not exceeding 0 colonies in 100 mL of water samples.



(A) Negative results: No gas bubbles were observed, and there was a color change in the media.
(B) Positive results: Gas bubbles were observed, and the color of the media became cloudy.

Figure 1: Observation results of MPN method

Table I: MPN results

Sample Code	Number of Positive Tubes			MPN Value per 100mL	Sample Code	Number of Positive Tubes			MPN Value per 100mL
	3x10mL	3x1mL	3x0,1mL			3x10mL	3x1mL	3x0,1mL	
SG-1	3	1	1	58	SG-22	3	3	2	438
SG-2	3	2	0	76	SG-23	0	0	0	0
SG-3	2	2	1	26	SG-24	3	3	2	438
SG-4	2	2	2	31	SG-25	0	1	0	3
SG-5	3	2	1	95	SG-26	1	0	0	4
SG-6	3	3	1	271	SG-27	3	1	0	46
SG-7	3	3	2	438	SG-28	3	3	1	271
SG-8	3	1	0	46	SG-29	3	1	0	46
SG-9	3	3	2	438	SG-30	3	3	1	271
SG-10	3	0	0	29	SG-31	3	0	1	39
SG-11	3	0	0	29	SG-32	1	0	0	4
SG-12	2	3	1	33	SG-33	3	3	1	271
SG-13	3	3	3	1898	SG-34	1	0	1	7
SG-14	3	3	3	1898	SG-35	1	0	0	4
SG-15	3	2	0	76	SG-36	3	1	0	46
SG-16	3	2	1	95	SG-37	3	3	0	190
SG-17	3	1	0	46	SG-38	0	0	0	0
SG-18	0	0	0	0	SG-39	3	3	2	438
SG-19	2	2	3	37	SG-40	3	0	0	29
SG-20	3	2	2	116	SG-41	2	0	0	10
SG-21	2	3	1	33	SG-42	3	3	3	1898

Meanwhile, SCA media in this study was used to identify enteropathogenic bacteria in dug well water samples.Table II shows that most of the dug well water samples contained enteropathogenic bacteria, namely 29 samples (69%). Enteropathogenic bacteria identified on SCA media are *Escherichia coli* and *Salmonella sp.* Identification of *E. coli* is indicated by the growth of blue colonies on SCA media. While *Salmonella sp.* is indicated by the growth of magenta-colored colonies. The results of bacterial identification on SCA media are shown in Figure 2.

Table II: SCA Result

SCA Media Result	Number (n)	Percentage (%)
Bacteria present	29	69.0
No bacteria	13	31.0
Total	42	100

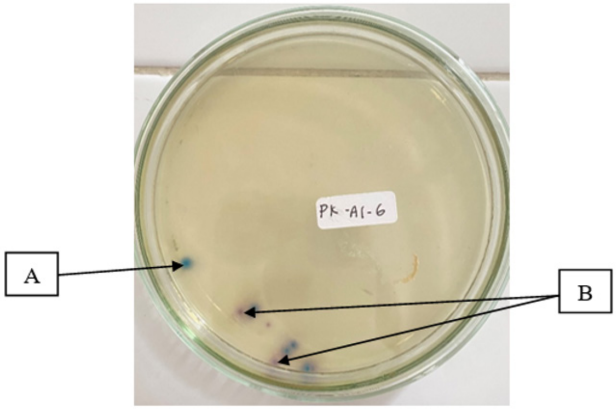


Figure 2: Identification results of SCA media. (A) Blue color on *Escherichia coli* colonies; (B) Magenta color on *Salmonella sp.* colonies

The results of identification using SCA media found the majority of enteropathogenic bacterium types found in dug well water samples, namely *E. coli* and *Salmonella sp.* in 18 samples (62.1%). There were only 5 samples (17.2%) for *Salmonella sp.* and 6 samples (20.7%) for *E. coli*. The distribution of enteropathogenic bacterium types found in the dug well water samples can be seen in Table III.

Table III: Distribution of enteropathogenic bacteria types

Type of *Enteropathogenic* Bacteria	Number (n)	Percentage (%)
Escherichia coli	6	20.7
Salmonella sp.	5	17.2
Escherichia coli & *Salmonella sp.*	18	62.1
Total	29	69

DISCUSSION

In Indonesia, waterborne illnesses have long been a problem for public health. A considerable danger of drinking water pollution was evident in Jember based on the town's sanitary condition and clinical data. In Jember, this study evaluated the drinking water quality from the reservoir to consumers' containers as well as the related risk factors.The bacteriological quality of dug well water in this study was assessed using two methods, namely the MPN method and SCA media. The use of the MPN method aims to determine the concentration of coliform bacteria in dug well water samples so that the MPN index will be known as an estimate of the number of coliforms in the sample which is useful for determining the feasibility of water quality based on quality standards. SCA media is used to identify the type of enteropathogenic bacteria found in dug well water samples. Enteropathogenic bacteria are bacteria that can cause digestive tract disorders.

The assessment of coliform bacteria concentration to determine the MPN value in this study used a three-tube dilution series. The results showed that the majority of dug well water quality in Jember District has not met the quality standards, as much as 92.9% (n=39). These results are in line with research conducted by Sari et al. in 2022 which stated that out of 31 dug wells, 28 (90.3%) dug wells contained coliform bacteria (14). This contradicts another study by Ratumbanua et al. in 2021, which showed that only 13.3% (n=4) of dug well water samples did not meet the requirements (15). Another study in Bangladesh by Parvez et al. in 2016 showed that out of a total of 53 samples of dug well water, 81.2% were contaminated with coliform bacteria (16).
Based on the Ministry of Health Regulation Number 2 of 2023 concerning the Implementation Regulation of Government Regulation Number 66 concerning Environmental Health, the maximum levels of total coliform and *E. coli* bacteria for drinking water quality parameters, hygiene, and sanitation are 0 colonies per 100 mL (17). The observation data showed that only 7.1% (n=3) of the dug well water samples in Jember District met the quality standard of 0 colonies. The MPN index of the dug well water samples in this study varied, with the highest value being 1898 MPN per 100 ml. In line with these results, a study by Ahmed et al. in 2015 in Dhaka, Bangladesh showed that all water samples (n=75) were contaminated by bacteria that can ferment lactose, with 16 samples of which showing MPN index results >2400 MPN per 100 mL and only 2 samples showing a low MPN index of 2 MPN per 100 mL (18). Another study by Shakya et al. in 2021 showed results contaminated with coliform bacteria with indices ranging from 4 to 10 MPN per 100 mL in 30 samples (37.5%) of a total of 160 samples (19).

The results of this study indicate that descriptively most of the water quality of the dug wells in Jember Regency, which are located adjacent to landfills, public cemeteries, slaughterhouses, and farms, still does not meet the quality standards for bacteriological water quality. Therefore, there is still a need for behavior to treat dug well water so that it is suitable and safe for consumption. One method that is widely used to treat water to make it suitable for use is by filtering and boiling (20). With a good water management process, the spread of diseases caused by water contamination can be reduced.

Observation data showed that the majority of dug well water samples located close to landfills, public cemeteries, slaughterhouses, and farms contained enteropathogenic bacteria, with a percentage of 69% (n=29). The enteropathogenic bacteria identified in this study were *E. coli* and *Salmonella sp.* A total of 62.1% (n=18) of dug well water samples that were positive for enteropathogenic bacteria were identified as containing both bacteria. Meanwhile, 17.2% (n=5) of the dug well water samples only contained *Salmonella sp.*, and 20.7% (n=6) only *E. coli*. This is in line with research conducted by Oktavianto et al. in 2016, which stated that of the 7 water samples, 100% contained *Salmonella sp.* and 57.1% (n=4) of other samples contained *E. coli* bacteria (21). Another study by Padeniya et al. in 2019 stated that 100% of well water samples (n=40) were contaminated with *E. coli* bacteria and another 8% contained *Salmonella sp.* (22). Based on Indonesian National Standard (SNI) Number 1-3553-2006, the maximum limit for the number of *E. coli* is <2 MPN per 100 mL and negative per 100 mL for *Salmonella sp.* (21). The results of this study show that descriptively the well water samples located adjacent to the landfill, public cemeteries, slaughterhouse, and farms taken and observed using SCA media show the results that most of the *E. coli* and *Salmonella sp.* were identified which do not all meet the standards.

CONCLUSION

The present research showed the bacteriological quality of dug well water in Jember Regency, which was measured using the MPN method and SCA media, showed that *Escherichia coli* and *Salmonella sp.* were identified with a percentage of 20.7% for *E. coli*, 17.2% for *S. typhi*, and 62.1% for these two bacteria. It was only found that 7.1% of dug well water still met quality standards. These findings suggest the importance of reducing or inactivating *Escherichia coli* and *Salmonella sp.* before for cooking, eating, and drinking.

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