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Microbiological quality of drinking water from transport jerrycans filled using public funnels at water supply points in the Atlantique department, southern Benin (West Africa)

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Abstract

To access potable water is vital for the people. In the supply chain, some populations sometimes use a public funnel to fill their jerrycans for transporting water from the source to households. This practice is a source of microbial contamination, which this study investigated over a three-year period. The hydrogen sulfide method was employed to detect microbial contamination in the water from some jerrycans with the community's participation. *Escherichia coli* and other coliforms indicating fecal contamination of the water in the transport jerrycans were analyzed at the microbiology laboratory. This aim was achieved by examining 216 samples of water from jerrycans filled using public funnels for drinking water according to ISO 19458: 2006. 41.7% of the samples tested positive for microbial contamination in 2021, while in 2023, the contamination rate increased to 50%. Logistic regression showed a statistically significant annual increase of 4% in the probability of detecting *Escherichia coli* (A = 0.039; OR = 1.040; p = 0.000). The presence of coliforms other than *Escherichia coli* also showed statistically significant variation, although the impact on detection probability was marginal (A = 0.000; P = 0.000). The bacteria found in the water contained in jerrycans filled with a public funnel cause an infectious risk to human health. Continuous monitoring of microbial contamination trends in household drinking water with a rigorous approach is essential for public health protection.

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Introduction

Access to quality drinking water is essential for public health, especially in rural areas where supply infrastructure is often limited (WHO, 2017). The drinking water supply chain includes both the collection and transportation of water from the source to households. Various containers, including jerrycans, are used for water collection and transport. These jerrycans are sometimes filled using public funnels that are exposed to open air for easy and quick access by the community This practice can introduce dust into the collected water through the uncovered funnels, posing risks of microbiological contamination (Alves *et al.*, 2021).

This microbiological contamination can be caused by several microorganisms, including *Escherichia coli*, which is used as an indicator of fecal contamination in water (WHO, 2017; Nowicki *et al.*, 2021). United Nations Sustainable Development Goal 6, by ensuring access to safe drinking water for all, also indicates the monitoring of fecal organisms as an indicator to measure the proportion of the population with access to drinking water that meets standards (United Nations, 2015).

The microbiological quality of drinking water contained in transport containers can be analyzed in microbiology laboratories or in households with hydrogen sulfide test kits. The hydrogen sulfide test, developed by Manja et al. (1982), has undergone various improvements worldwide. The simplicity of the hydrogen sulfide test makes it suitable for populations in rural and remote areas (Gupta et al., 2008). Although practical and simple for determining the microbiological quality of drinking water, the hydrogen sulfide test is not recommended for routine monitoring due to its low specificity and the absence of systematic standardization studies (OMS, 2002; UNICEF, 2008). While the hydrogen sulfide test alerts households to the state of contaminated water. the lack of specificity in identifying fecal microorganisms necessitates a confirmation test in a microbiology laboratory for accurate details (Chuang et al., 2011; Moses et al., 2023; Islam et al., 2017).

Like other manufacturers, the Water and Food Quality Control Laboratory (LCQEA) of the Ministry of Health of Benin, with the support of the German Agency for International Cooperation (GIZ), has improved the performance of the hydrogen sulfide test to determine water quality within populations.

The objective of the study was to determine the microbiological quality of drinking water from transport jerrycans filled using public funnels at water supply points.

The research focuses on the use of the hydrogen sulfide test within the population, with confirmation in a microbiology laboratory to obtain accuracy and specifications. By adopting this approach, the present research aims to contribute to raising awareness about the sanitary condition of drinking water in households using a simple hydrogen sulfide test method, in order to improve water safety in rural communities.

Materials and methods

An analytical study was conducted on water from jerrycans filled using public funnels at sales points in the Atlantique department, southern Benin (West Africa). The Atlantique department is one of the smallest of the twelve departments in Bénin republic, with a total area of 3.233 km². It stretches nearly 100 km from the coast inland. Despite its size, it has the largest population among the departments of Bénin republic, with 1398229 inhabitants (INSAE, 2013).

Primarily characterized by a subequatorial climate, the Atlantique department experiences two dry seasons and two rainy seasons. However, access to potable water remains a significant challenge for its inhabitants. Approximately 54.2% of households have access to potable water (INSAE, 2013).

The objective of the study was to identify the presence of bacteria (*Escherichia coli* and coliforms other than *E. coli*) using two methods. Bacteriological tests were carried out at the Laboratory of Food Microbiology, Ministry of Health (Bénin republic). The study period spanned from 2021 to 2023, focusing on the last week of December, which coincides with the annual festive period when the demand for water peaks.

Sample collection

Seventy-two samples were collected per year, with a total of 216 samples of water from jerrycans filled using public funnels for drinking water (Fig. 1A-D). From each jerrycan, two samples were collected: one for the hydrogen sulfide (H₂S) method and the other for analysis in the microbiology laboratory. All microbiological samples were taken in sterile Whirl-Pak bags. Immediately after collection, the samples were packed with cooler packs in a cooler and sent to the laboratory (ISO 5667-3, 2004).



Fig. 1A-D. A. Public funnel exposed to open air for easy and quick access by the community, B. Public funnel in use for filling a water jerrycan at the supply source, C. Public funnel made from a polyethylene bottle in use for filling a jerrycan, D. Used plastic bottle serving as a funnel for filling a jerrycan

Sample processing

In this study, two analytical methods were used. The first method was the hydrogen sulfide test, conducted in the field within the community. The second method was performed in the microbiology laboratory (ISO 9308-1, 2014).

Hydrogen sulfide method in the community

The vials for the hydrogen sulfide test for detecting microbial contamination used in this study were produced by the LCQEA with support from GIZ. They come in the form of a tube of about 15 milliliters containing a paper reagent, to which 10 ml of the sample to be tested is added (Fig. 2A). The result appears after three days. In the case of a negative test, the contents of the tube remain yellow (Fig. 2B). For a positive reaction, the reagent in the tube turns black and emits a foul odor of rotten eggs (Fig. 2C). In this case, the microorganisms present in the sample have reduced the sulfur to hydrogen sulfide, forming a black iron sulfide precipitate in the presence of ferrous iron.

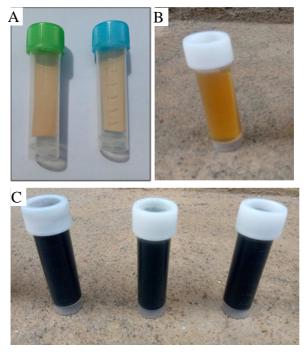


Fig. 2A-C. A. Hydrogen sulfide test vials for detecting microbial contamination, B. Hydrogen sulfide test vials indicating a negative test with a yellow precipitate for non-contaminated water, C. Hydrogen sulfide test vials indicating a positive test with a black precipitate for contaminated water

Analysis in the microbiology laboratory

The filtration of 250 ml of the sample through a membrane filter retained the organisms, and the

membrane filter was then placed on chromogenic coliform agar (RAPID' *E. coli* 2) (ISO 9308-1, 2014). RAPID' *E. coli* 2 is a selective chromogenic agar used for the direct enumeration, without confirmation, of *Escherichia coli* and other coliforms in water. The membrane filter was incubated at (36 ± 2) °C for (21 ± 3) 3 hours (ISO 9308-1, 2014).

Counting positive colonies: for Coliforms other than *E. coli* « beta-D-galactosidase (GAL) +/ P-D-glucuronidase (GLUC) -», form blue to green colonies, whereas, specifically, *E. coli* « beta-D-galactosidase (GAL)+/ P-D-glucuronidase (GLUC) +» form violet colonies. From the number of confirmed colonies counted on the filter membrane, the total number of coliforms was determined (ISO 9308-1, 2014).

Statistical analysis

The results of bacteriological analyses were compared with the WHO Guideline Values for the quality of human drinking water. All statistical analyses were performed using SPSS Statistics 21 software. The Spearman Correlation Test was employed for the variables of interest (Funnel Filling Practice, Contamination Detected by Hydrogen Sulfide Method, Presence of *E. coli*, Presence of Coliforms other than *E. coli*).

The Chi-Square Test was utilized to analyze the relationships between categorical variables. The Analysis of Variance (ANOVA) was used to compare the means of several groups (2021, 2022, and 2023) to determine if the differences observed in the means of the variables of interest are statistically significant. A logistic regression was conducted to identify the significant factors influencing the presence of *E. coli* and coliforms other than *E. coli* in the water samples.

Results and discussion

Table 1 indicated that the hydrogen sulfide method used in this study identified microbial contamination in some water jerrycans filled via public funnels (44.9%), with variation from year to year. Moses *et al.* (2023) also used the hydrogen sulfide method to identify microbial contamination in rainwater collected from rooftops. The alteration of the microbiological quality of drinking water can pose potential public health risks (Ling et al., 2018). Water samples contaminated with E. coli in Bangladesh have been associated with diarrheal diseases in children, highlighting the importance of improving drinking water quality for public health (Luby et al., 2015). Although some correlations are not established in Table 2 (Rho=0.000; p=0.000), it is crucial to maintain rigorous management measures to prevent other contamination pathways and, in a holistic approach, protect the quality of drinking water (Ling et al., 2018). Table 3 showed a statistically significant association between funnel filling practices and microbial contamination detected by the hydrogen sulfide method (χ^2 =14.000; p=0.000). The same applies to the relationship between funnel filling practices and the presence of *E. coli* (χ^2 =49.000; p=0.000), as well as the relationship between funnel filling practices and the presence of coliforms other than *E. coli* (χ^2 =24.000; p=0.000). These findings encourage the reinforcement of hygiene principles in the household drinking water supply chain. The presence of E. coli in water samples from jerrycans filled with public funnels (Table 4) significantly varied from 2021 to 2023 (F = 0.026; χ^2 = 0.052; p = 0.000).

Table 1. Contamination detected by hydrogen sulfide method

Year of sample collection	Positive results
2021	30 (41.7%)
2022	31 (43.1%)
2023	36 (50.0%)
Total	97 (44.9%)

This significant variation was also observed for the presence of coliforms other than *E. coli* detected in water samples from jerrycans filled with public funnels (F = 2.000; p = 0.000).

These increases (Table 4) indicate a growing risk of microbial contamination over the years. It is essential to implement stricter monitoring measures to improve hygiene in household water supply, preventing community infections.

Table 2. Spearman correlation test results

Pairs of variables	Correlation	Significance	Conclusion
	coefficient (Rho)	(p-value)	
Funnel filling practice and contamination detected by hydrogen	0.000	0.000	No correlation
sulfide method			
Funnel filling practice and presence of <i>E. coli</i>	0.000	0.000	No correlation
Funnel filling practice and presence of coliforms other than E. coli	0.000	0.000	No correlation
Contamination detected by hydrogen sulfide method and presence	0.000	0.000	No correlation
of E. coli			
Contamination detected by hydrogen sulfide method and presence	0.000	0.000	No correlation
of coliforms other than <i>E. coli</i>			

Table 3. Analysis of relationships

Pairs of variables	Pearson's Chi-square	p-value	Conclusion
Funnel filling practice and contamination detected by hydrogen sulfide method	14.000	0.000	Significant association
Funnel filling practice and presence of <i>E. coli</i>	49.000	0.000	Significant association
Funnel filling practice and presence of coliforms other than <i>E. coli</i>	24.000	0.000	Significant association
Contamination detected by hydrogen sulfide method & presence of <i>E. coli</i>	68.000	0.000	Significant association
Contamination detected by hydrogen sulfide method & presence of coliforms other than <i>E. coli</i>	103.000	0.000	Significant association

Table 4. Comparison of means across different years

Variables	Test	Test statistic	p-value	Conclusion
Contamination detected by hydrogen	ANOVA	F = 0.000	0.000	No significant difference
sulfide method				
Presence of <i>E. coli</i>	ANOVA & Kruskal-	F = 0.026,	0.000	Significant difference
	Wallis	Chi ² = 0.052		(validated by Kruskal-Wallis)
Presence of coliform other than E. coli	ANOVA	F = 2.000	0.000	Significant difference

Table 5. Predicting the probability of microorganism presence

Microorganisms	Regression coefficients (B)	Statistical significance (p-value)	Odds ratio (Exp(B))
Presence of E. coli	0.039	0.000	1.040
Presence of coliforms other than E. coli	0.000	0.000	0.000

In this study, each additional year, as shown in Table 5, brings a statistically significant 4% increase in the probability of detecting *E. coli* (A = 0.039; OR = 1.040; p = 0.000). Year over year, there is a statistically significant presence of coliforms other than *E. coli*, although the annual impact on the probability of detecting these microorganisms remains marginal or constant (A = 0.000; OR = 0.000; p = 0.000). Therefore, continuous monitoring of contamination trends using improved methods and strengthening water quality management policies in households are necessary to protect public health.

Conclusion

Microbial contamination of drinking water in jerrycans filled with public funnels is a proven fact by the hydrogen sulfide analysis method, conducted within the community. The variations in microbial contamination of water observed between 2021 and 2023 highlight a growing infectious risk, necessitating increased attention to the management of drinking water supplies. The significant annual increase in the probability of detecting *E. coli* indicates the need for rigorous interventions to prevent community infections. A holistic approach to the continuous awareness of communities on hygiene at all levels of the drinking water supply chain is crucial to combat

microbial contamination of drinking water and its potential impacts on human health.

References

Alves A, Viveiros C, Lopes J, Nogueira A, Pires B, Afonso AF, Teixeira C. 2021. Microbiological contamination in different food service units associated with food handling. Applied Sciences **16**, 1-10.

Chuang P, Trottier S, Murcott S. 2011. Comparison and verification of four field-based microbiological tests: H2S test, Easygel®, Colilert®, Petrifilm®. Journal of Water, Sanitation and Hygiene for Development 1, 68-85.

Gupta SK, Sheikh MA, Islam MS, Rahman KS, Jahan N, Rahman MM, Hoekstra RM, Johnston R, Ram PK, Luby S. 2008. Usefulness of the hydrogen sulfide test for assessment of water quality in Bangladesh. Journal of Applied Microbiology **104**, 388-395.

Institut National de la Statistique et de L'Analyse Economique (INSAE). 2013. Cahier des villages et quartiers de ville du département de l'Atlantique, Recensement Général de la Population et de l'Habitation du Bénin-2013.

International Standard Organization (ISO) 19458. 2006. Qualité de l'eau – Echantillonnage pour analyse microbiologique.

Islam M, Ercumen A, Naser AM, Unicomb L, Rahman M, Arnold BF, Colford JMJr, Luby SP. 2017. Effectiveness of the hydrogen sulfide test as a water quality indicator for diarrhea risk in rural Bangladesh. American Journal of Tropical Medicine and Hygiene **97**, 1867-1871.

Ling F, Whitaker R, LeChevallier MW, Liu WT. 2018. Drinking water microbiome assembly induced by water stagnation. ISME Journal **12**, 1520-1531. Luby SP, Halder AK, Huda TM, Unicomb L, Islam MS, Arnold BF, Johnston RB. 2015. Microbiological contamination of drinking water associated with subsequent child diarrhea. American Journal of Tropical Medicine and Hygiene **93**, 904–911.

Manja KS, Maurya MS, Rao KM. 1982. A simple field test for the detection of fecal pollution in drinking water. Bulletin of the World Health Organization **60**, 797–801.

Moses A, Ramírez-Andreotta MD, McLain JET, Obergh V, Rutin E, Sandhaus S, Kilungo AP. 2023. The efficacy of hydrogen sulfide (H2S) tests for detecting microbial contamination in rooftop-harvested rainwater. Environmental Monitoring and Assessment **195**, 1-11.

Nations Unies. 2015. Objectif de Développement Durable 6: Assurer l'accès de tous à l'eau et à l'assainissement. New York: Nations Unies. https://www.un.org/sustainabledevelopment/fr/wate r-and-sanitation/

Nowicki S, deLaurent ZR, de Villiers EP, Githinji G, Charles KJ. 2021. The utility of *Escherichia coli* as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. PLOS ONE 16, 1-23.

Organisation mondiale de la Santé (OMS). 2002. Evaluation of the H₂S method for detection of fecal contamination of drinking-water. Genève, Suisse: OMS. Retrieved from https://iris.who.int/handle/10665/205188

Saxena G, Bharagava R, Raj A. 2015. Microbial indicators, pathogens and methods for their monitoring in water environment. Journal of Water and Health **13**, 319-339.

United Nations Children's Fund (UNICEF).2008. Handbook on water quality. New York:UNICEF.Retrievedhttps://www.unicef.org/media/91301/file/Handbook-Water-Quality.pdf

World Health Organization (WHO). 2017. Guidelines for drinking-water quality. Geneva, Switzerland: WHO. Retrieved from https://www.who.int/publications/i/item/978924154 9950