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Faecal coliforms and pathogenic bacteria in borehole water and household storage in Ekalakala Ward, Machakos County, Kenya

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Article Info

KEYWORDS

Borehole water

Water contamination

Diarrhea

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Faecal coliforms

Sanitation

ABSTRACT

Quality of drinking water is very vital to public health. Diarrhea can be caused by poor hygiene practices, inadequate sanitary action and unsafe drinking water. Globally, 1,000 children die annually due to controllable sanitation - related diarrhea diseases. Furthermore, Africa has been classified as the leading continent in cholera disease due to lack of access to safe clean water and basic sanitation. Kenya is a water scarce country in Africa leading to drilling of many boreholes in the country. Almost all the households in Ekalakala ward, Machakos County used borehole water for various activities. This study aimed to determine faecal and pathogenic coliforms in borehole water at source and households in Ekalakala ward, Machakos County. Mixed methods research design was adopted. The most probable number (MPN) technique was used to identify faecal coliforms (Escherichia. coli). Other pathogenic bacteria include Vibrio cholerae and Salmonella species and were isolated using thiosulfate citrate bile salts and deoxycholate citrate agar respectively. MPN results showed that most of the samples were contaminated with faecal coliforms with a mean value of 171.3 MPN/100 ml of water. The proximity of boreholes to pit latrines was assessed using a checklist and confirmed that there was moderate positive correlation of (r) = 0.45. Borehole water in Ekalakala ward was contaminated with bacterial coliforms and faecal coliforms. Water treatment before use in addition to, education on proper water storage methods by community health providers should be emphasized.

Introduction

Globally, 4.2 billion still require basic sanitation and clean water (WHO, 2015). The World Health Organization (WHO) adopted 17 Sustainable Development Goals (SDGs) (WHO 2015). These goals were to act as roadmaps for uprooting hunger, poverty, and obtaining clean water and basic sanitation by 2030. However, microbial water contamination still remained a major public health problem in resource-poor sub-Saharan Africa (Lutterodt et al., 2018).

Sustainable Development Goal six focuses on sustainable management of clean water and sanitation for all, and the first indicator of clean water is to achieve universal and equitable access to safe and affordable drinking water for all by 2030. The achievement of SDG 6 would immensely support SDG 3: Universal health for all by achieving indicator 3.9, which strives to reduce deaths from hazardous chemicals, air, water, soil pollution, and contamination.

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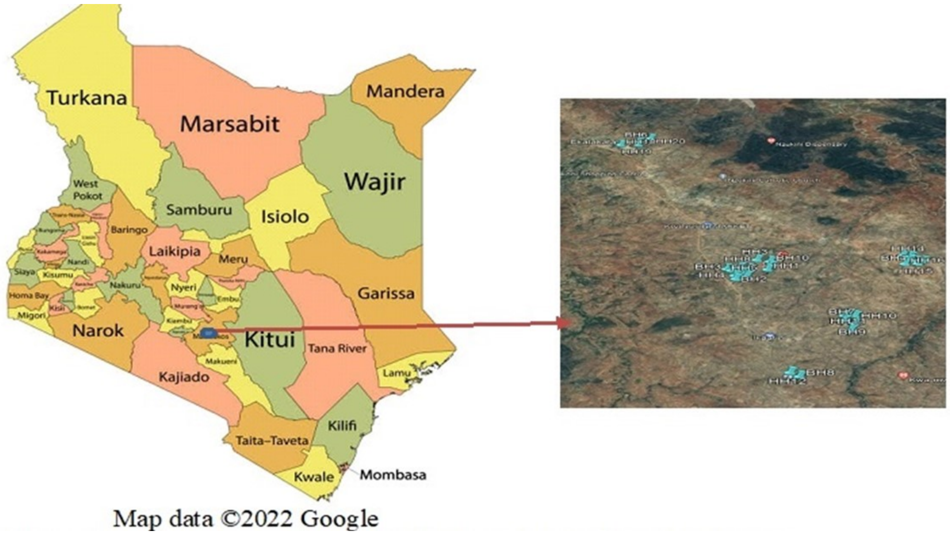

Figure 1: *A map of Kenya showing Ekalakala Ward with sampling sites*

Diarrhea was believed to be caused by inadequate sanitary action and unsafe drinking water (Mwashumbe, 2019). Moreover, the scarcity of access to clean drinking water had emerged as a pivotal factor perpetuating the cholera crisis across the continent (Livingston, 2021).

Reports from Kenya indicated that 59% of population had access to safe drinking water while, only 29% had access to basic sanitation (UNICEF, 2020). Clean water, an essential resource for maintaining personal and communal hygiene, is a precious rarity in many regions, perpetuating a cycle of infection and exacerbating the severity of cholera outbreaks

A study in the Marigat urban center in Kenya indicated that all drinking water points in the area were contaminated with microbes such as E. coli and Salmonella species and there was a need to curb water-borne diseases through public health awareness campaigns on the management of household water (Osiemo et al., 2019).

According to Itoya (2015), 63 % of children aged five years and below had experienced diarrhea in the neighboring region to Machakos County due to poor water quality. A related study by Sila (2019) highlighted that one person died from cholera while four got hospitalized during cholera outbreak in Yatta, Machakos County. The health systems may not be able to attend to high number of diarrhea reported cases therefore exceeding the health system capacity

The strategic selection of Ekalakala Ward as the epicenter of the study was guided by a multifaceted rationale. First, the decision was motivated by the absence of any prior investigations into the microbial quality of borehole water within this specific region. This depth of research underscored the novelty and significance of the study, potentially shedding light on uncharted aspects of water quality within this unique context. Additionally, the overwhelming reliance of approximately 90% of the community on borehole water for domestic needs (Elija & Ondiyo, 2019). This pronounced reliance emphasized on the practical relevance and implications of the study's findings, as they directly impact the health and well-being of a substantial portion of the population.

Method

Study area

The study area was Ekalakala Ward in Machakos County, Kenya. Residents of the area use seasonal rivers and wells, which eventually dry up and are left with boreholes as the main source of water for drinking and other activities. The study area was generated using Google Maps with sampled boreholes and households appearing as pins.

Study design

The study used mixed methods research design by collecting water samples, analyzing them in laboratory and assessing distances of boreholes from pit latrines by observation method.

Sampling procedure

Most boreholes are located in public institutions, such as schools, markets, roads, and chief camps. Samples were collected from boreholes and households which obtained water from those boreholes. A 70% ethanol was used to sterilized the hands before collecting water from both boreholes and households. Water was stored in cold ice boxes and thereafter analyzed in the laboratory for microbe coliforms using the most probable number and differential tests for E. coli, Vibrio cholera and Salmonella species

Sample size

Sample size determination was done using Krejcie and Morgan table (Krejcie & Morgan, 1970). A total of 20 samples was collected, 10 samples from boreholes and 10 from households. Sub sampling was done in the laboratory totaling to 80 samples. 20 samples for MPN, E. coli, Salmonella species and V. cholerae tests respectively.

Sampling technique

This study used simple random sampling method. The population was first listed, assigned numbers, found random number and finally selection of sample. The observation method, measuring rob together with checklist were used to note proximity of pit latrines to boreholes.

Laboratory analysis

Faecal contamination using most probable number: The borehole water samples from source and households were subjected to the three stages of the MPN test; Presumptive laboratory test whereby lactose broth was prepared and autoclaved for any microbes. The single-strength lactose broth was dispensed into 10 test tubes containing 10 ml of lactose broth while the double-strength lactose broth was also dispensed into the remaining five tubes in 20 ml of lactose broth. The series consisted of 15 tubes of three groups, each of five tubes of specified medium. The five tubes contained 10 ml double strength broth, and the remaining 10 tubes contained 9 ml single strength broth to increase sensitivity. Water samples were placed in five tubes each with a Durham tube, flamed mouth, inoculated, and labelled according to the water source and amount of water inoculated. The inoculated broths were incubated at 37oC for 24 h, after which the tubes that produced both gas and acid (turned turbid) were counted and recorded. The results were then compared to the probability table to determine the most probable number of faecal coliform bacteria in 100 ml of water samples at a 95% confidence level (Williams et al., 2020). Two positive tubes from each set were used for the confirmatory testing. Confirmatory test was done by testing the positive tubes from presumptive test using selective and differential media; Eosin Methylene Blue and incubated for 24 hours at 370c. The plates that formed dark centers and green metallic sheen were recorded. Completed test was carried out by scooping isolated colonies from the confirmatory test plates by flamed wire loop and inoculated into a lactose broth tubes at 370 C for 24 hours and later streaked on a nutrient agar slant in triplicates. The streaked slants were incubated for 24-48 hours at 370 C. Tubes showing acid and gas in the lactose were suggestive indication of complete test for E. coli.

Pathogenic bacteria: Differential media were used to enumerate these bacteria. In Salmonella spp, 1 ml of each of 20 sampled water were place in test-tubes containing selenite broth and incubated at 370 C for 24-48 hours. The tubes that turned pink were inoculated in deoxycholate citrate agar (DCA) in petri dishes in triplicates then incubated at 370 C for 24-48 hours. The positive plates formed colonies with black sports and some formed colorless colonies. Vibrio cholerae; 1 ml from each 20 sampled water was added to peptone water which is colorless and incubated tubes at 370 C for 24 hours. The tubes that were turbid were selected and inoculated to thiosulphate citrate bile salts (T.C.B.S) media in petri dishes then incubated at 370 C for about 24 hours. The positive plates formed yellow colonies.

Ethical considerations

Approval to carry out study was done by the Meru University of Science and Technology Institutional Research and Ethics Review Committee (MIRERC). Approval Ref NO: MU/1/39/28 Vol.2 (139). Date 10th May, 2023. A consent letter to carry out the research was given out by Ekalakala ward administrator.

Proximity of pit latrines to the borehole: A checklist was generated for the distance from the borehole to the pit latrines. A threshold of 30 meters was used. Measuring rod was used to determine the distances. Boxes were ticked for the distances less or more than 30 meters and a record of exact distance recorded in a dairy. The boreholes with less than 30 meters distances were expected to be more contaminated than those with more than 30 meters.

Data analysis

Microbial analysis was performed in the laboratory for 20 samples using the most probable number (Williams et al., 2020) and total coliform forming units. Tables were generated for the most probable number and total coliform forming unit results. Three organisms were targeted: E. coli, Salmonella spp and Vibrio cholerae. The results of the three bacteria on differential media and biochemical tests are separately recorded in the tables. Data from the most probable number, differential media, and biochemical tests were entered into Microsoft Excel V 2010 and analyzed using the Statistical Package for Social Sciences (SPSS) version 27 for descriptive statistics.

Results

Most probable number technique

The results of twenty samples were presented in tables. The tubes which produced gas were compared to the MPN standard chart at 95% confidence level. The MPN mean value was at 171.35MPN/100ml of water. Borehole 5 and 6 were the most contaminated with borehole 3 and 4 being the least contaminated. Table 1 was a presentation of MPN results with an interpretation whether the water was satisfactory or not for human consumption.

Sample	MPN/100ml	Category	Interpretation
B1	170	D	Unsatisfactory
B1H1	2400	D	Unsatisfactory
B1H2	2400	D	Unsatisfactory
B2	170	D	Unsatisfactory
B2H1	170	D	Unsatisfactory
B3	< 2	A	Excellent
B3H1	22	D	Unsatisfactory
B3H2	2400	D	Unsatisfactory
B4	2	A	Excellent
B4H1	110	D	Unsatisfactory
B5	2400	D	Unsatisfactory
BH1	2400	D	Unsatisfactory
B6	2400	D	Unsatisfactory
B6H1	2400	D	Unsatisfactory
B7	540	D	Unsatisfactory
B7H1	2400	D	Unsatisfactory
B8	2	A	Excellent
B8H1	49	B	Satisfactory
B8H2	1600	D	Unsatisfactory
B8H3	110	D	Unsatisfactory

Table 1: *Most probable number table*
Key: B stands for borehole while H household.

The data of coliforms ranged between 0-2400MPN/100 ml of water. Three samples from boreholes had less than 2MPN/100 ml of water and four samples had a range of 170-240MPN/100 ml of water. From households, eight samples had faecal coliforms of 240MPN/100 of water, indicating that the contamination level increased in the households. The results showed that only one of the samples did not produce gas, while the remaining samples not only produced gases in large volumes but also turned turbid.

Pathogenic bacteria targeted

The three targeted organisms were E. coli, Salmonella species and Vibrio cholerae coded as 1,

Microorganisms		Frequency	Percent
Valid		5	22.7
	E. coli	4	18.2
	1,2,3	5	22.7
	1,3	2	9.1
	2,3	1	4.5
	V. cholera	5	22.7
	Total	**22**	**100**

Table 2: Target organisms' frequency
The codes are represented as 1 for E. coli, 2 for Salmonella and 3 for V. cholerae.

2, and 3, respectively (table 2). Twenty samples were tested for all the three organisms. E. coli and V. cholera solely contaminated 18% and 23% of the samples respectively while 36% of the samples were contaminated by either two or three pathogenic microorganisms.

Proximity of latrines to boreholes

The results for relationship between faecal borehole water contamination, and the distance of these boreholes from the pit latrines was determined using excel 2010 at 95% confidence level. Table 3 was result from regression and correlation(r) was obtained from multiple R. The results showed there was a moderate positive correlation at r (6) = 0.45

Regression Statistics	
Multiple R	0.452873
R Square	0.205094
Adjusted R Square	0.07261
Standard Error	1018.38
Observations	8

Table 3: A table of relationship between borehole faecal contamination and pit latrine distances.

Table 4 was that of Analysis of variance (ANOVA). The P value was obtained from significance F which was at F = 0.259821. This value was higher than P = 0.05 and therefore value of P was P > 0.05. From the analysis, the results were not significantly different at P = 0.26.

Discussion

Twenty borehole water samples from Ekalakala ward were tested for faecal contamination. Only two of the 20 samples were potable. Ninty nine percent (99%) of the samples were contaminated with faecal coliforms. the boreholes in Ekalakala ward were deep boreholes some located close to pit latrines. Fidal et al. (2022) reported that boreholes in Pakistan were highly contaminated with E. coli and faecal coliforms. The high faecal coliforms could have been contributed by the close proximity of the latrines.

In the current study, water from deep boreholes and households was collected, transported and stored in plastic jerricans. These water samples were contaminated with high levels of faecal coliforms. Of the 20 samples, 11 (55%) contained E. coli. The trend could be explained by storage practices or the mishandling of samples during collection and analysis.

Dimza (2021), reported 11MPN/100 ml total coliforms from borehole water in Mai Idris polytechnic which related well with Adesakin et al., (2020), results of borehole water from Zaria, Nigeria which recorded 3.00 MPN/100 ml of water. These studies had lower levels of faecal, bacterial and total coliforms than current study at mean value of 171.3 MPN/100 ml of water. The phenomenon might be due to partial treatment of borehole water before collection in previous studies since the current study had no records of prior treatment.

Osei's study reported that faecal coliforms were present in 55% of the borehole water samples in Ghana by use of petrifilm method (Osei et., 2019). These results concurred with the current study which reported fifty-five percent (55%) E. coli coliforms in borehole water. The

	Df	SS	MS	F	Significance F
Regression	1	1605495	1605495	1.548064	0.259821
Residual	6	6222593	1037099		
Total	7	7828088			

Table 2: *ANOVA. The value of P = 0.26.*

water from households was glossily polluted with coliforms above 50 MPN/100ml of water in all households. Although the water at source was still satisfactory, further contamination could make it suspicious or glossily polluted. This unveiled the high contamination of boreholes with faecal coliforms regardless of location.

The Osei's study used petrifilm method to analyze the total and faecal coliforms since it was the most consistent method. On the hand, this study used the most probable number (MPN)technique to determine faecal coliforms. The most probable number because it was simple in its operation and had a high sensitivity (Niu & Zhang, 2022). The method was not very accurate and was time-consuming.

The current study assessed the proximity of boreholes from pit latrines. E. coli, Salmonella species and V. cholerae as some of water pathogenic contaminants isolated. It was a clear indication that boreholes can be contaminated by other microbes other than faecal coliforms (E. coli). Both E. coli and V. cholerae registered 55.0 % while Salmonella spp 40.0%. For concern, 40% of samples were contaminated with more than one bacterium organism.

According to Genter et al. 2022, 66% of borehole water samples from urban Indonesia were detected to have E. coli and boreholes surrounded by sanitary risk factors such as open defecation. The current study isolated E. coli, V. cholerae and Salmonella from borehole water and some of these boreholes were located near pit latrines. This study registered slightly lower results than those of Genter et al., 2022 maybe due type of sanitary facilities used in the area.

The three pathogenic organisms (E. coli, Salmonella spp and V. cholerae) were of public health interest. In Adamawa, Cameroon, boreholes which were close to pit latrines registered 61.19% contamination of both Salmonella spp and E. coli (Viban et al., 2021). The current study recorded 55.0% E. coli, 40% Salmonella and 55,0% Vibrio cholerae. Given that on 50% of boreholes had a distance less than 30, contamination was witnessed in all boreholes except one borehole.

These results were not unique to this study since other studies such that of Atnafe et al., (2017) reported isolation of E. coli and Salmonella spp from borehole water in Hawassa, Ethiopia. V. cholerae and E. coli were the dominating pathogens in borehole water and this explained the diarrhea prevalence within the area.

Ekalakala ward community mostly used ox- pull carts and since microbial contamination is caused by faecal matter from humans and animals, their faecal matter might have contributed to contamination. It can be deduced that, there could be other factors which might include but not limited to animal droppings, lack of regular borehole microbial management and proper alignment of boreholes.

However, while these studies offered invaluable insights, there remained a room for further empirical exploration. Other studies on proximity of boreholes to sanitary facilities and faecal contamination relationship reported similar cases of contamination. The empirical amalgamation of studies highlighted the paramount significance of proximity, sanitation practices, and disinfection in the realm of water contamination.

Conclusion

Borehole water at storage was highly contaminated by bacteria coliforms. Isolation of E. coli was an indication that borehole water was contaminated by human and animal excreta which could pose health challenges to the community. Distance of pit latrines to boreholes was a minor factor in borehole water faecal contamination.

Recommendations

Advice to the community on methods of water treatment before use such as chlorination and boiling. Public hygiene education by ministry of health to the members of community on proper water storage. Future research on boreholes pathogenic contamination in relation to leaking septic tanks.

Authors contributions

PM: Conceptualization, methodology design, proposal writing, provision of laboratory reagents and samples, investigation, formal analysis, thesis writing, and publication.

SW: Guided and corrected proposal writing, methodology, data analysis, and thesis writing.

EM: Guided and corrected proposal writing, data analysis, methodology, and thesis writing.

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Data Availability statement

The relevant data is included in this paper.

Competing interests

The authors have no competing interests in this research.

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