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BACTERIOLOGICAL QUALITY STATUS OF BOREHOLES WATER IN THARAKA NITHI COUNTY, KENYA

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ABSTRACT: *Water shortage that is caused by long droughts and higher cost of accessing piped water have forced many households in Kenya to opt for wells and borehole water. Where they are used, boreholes are exposed to contamination by pathogenic microorganisms from nearby toilets, defecation from domesticated animals and surface runoff. Despite of known health concerns of consuming contaminated water, many rural residents still consume borehole water whose portability is not determined and may not meet WHO standards. Thus, this study was conducted in Tharaka Nithi County to assess bacterial quality status of selected boreholes in Maara, Igamba Ngombe and Tharaka Constituencies between April to September 2019. A total of 108 water samples were collected from 36 boreholes. Boreholes in Mukothima within Tharaka area were not collected as the ones accessed were dry at the time of sampling. The samples were analyzed at Chuka University using most probable number methods, Plate count and bacteria species identified using selected biochemical methods. Data (MPN) for total and feacal coliform for different location of study and boreholes were log transformed (log10 (cfu+3) and compared using general linear method in SAS. Significance means were separated using least significant difference post hoc test in SAS version 9.4. Number of bacteria isolated from water samples were also compared using general linear model. Feacal coliform values exceeding WHO recommendation of (0 CFU/ 100 ml was observed in all borehole surveyed. Mean MPN value of 17.5/ 100 ml CFU was observed in Kawangware. Coliform bacteria E. coli, Bacillus spp, Klebsiella spp were isolated in all the water sampled. However, E. coli followed by Bacillus spp were higher compared to the rest of isolates. Occurrence of these bacteria in borehole water put the health of depended consumers at risk of infection.*

KEYWORD: Borehole Water, Quality, Bacteriological, Contamination, Tharaka Nithi, Kenya

INTRODUCTION

In Kenya, expenditure towards water projects by the National government in some counties has increased. However, long droughts, high poverty incidence, higher cost of accessing piped water, insufficient planning and managing limit access to water resources (Development Initiatives, 2018). Shortage of water has seen increased use of wells and boreholes as alternative water source. Boreholes and wells therefore provide water for drinking by human and livestock, irrigation and even for industrial processes (Onuorah *et al.*,

2019). However, water contamination remains a significant challenge on water quality. Thus, it has become harder to get clean, safe and portable water in most developing countries in Africa, Kenya included (Dara and Mishra, 2011; Idibie *et al*., 2018).

According to Water Resource Authority ([WRA], 2018), shortage of portable water in Kenya may worsen by the year 2030 due to degradation of its quality by human activity. Anthropogenic activities may cause ground water contamination where wastes are carried to water source (Boreholes) by flood splash, leachate from buried waste, latrine and septic tanks (Obot and Edi, 2012; Sebiawu *et al.,* 2014; Sila, 2019). Despite of eminent health concern about 1.5 billion global populations depend on untreated ground water (Palamuleni and Akoth, 2015; Idibie *et al*., 2018). Water consumed by the majority of rural residents may not meet WHO standards (Obioma *et al.*, 2017). Bacterial, Fungal, and parasites, physical and dissolved chemicals are some of the water contaminants and associated with illness and deaths (Babič*et al.,* 2017). Indeed, global mortalities associated with these contaminants exceeds 5 million people annually (Gleick, 2002; Ajobiewe *et al*., 2019).

Microbial contaminants of drinking water include enteric bacteria generally termed coliforms represented by *E. coli* as an indicator organism. O*ther microorganisms are Giardia lamblia and Cryptosporidium parvum* (Opara and Nnodim, 2014). Occurrence of Klebsiella, *Escherichia coli* and *Enterobacter bacteria* species in water may point at the existence of medically significance pathogens such as *Salmonella, Clostridium pafringen* and Protozoa (Jay *et al*., 2005; Anyamene and Ojiagu, 2014). Consumption of contaminated water with these pathogens results in diarrhea, giardiasis, dysentery, and gastroenteritis among other implications (Isikwue and Chikezie, 2014; Oludairo and Aiyedun, 2016). Infection by bacteria contaminants may be symptomized by stomach cramps, nausea, vomiting and fevers occurring within ten days upon consumption in water (Ugwuzor and Ifeanyi, 2015). Assessment of bacterial wells and borehole water quality in rural, semi urban and urban area is necessary to determine water portability, generate data for management and prevent illness and deaths that may result from water contamination. Thus, the current study was carried out to determine bacterial water quality of selected 'boreholes' in Tharaka Nithi County where borehole is depended upon for provision of water for domestic use.

METHODOLOGY

Study Area

Tharaka Nithi County lies between longitudes 37º 19' and 37º 46' East and latitude 000 07' and 000 26' South. The lowest altitude in the County is 600 m while the highest altitude is 5,200 m. Average annual rainfall received by the County is about 717 mm. Whereas the high altitude areas such as Chuka and Chogoria receive reliable rainfall, lower regions such as Kathwana and entire Tharaka region receive poorly distributed, unreliable and low rainfall. Temperatures in Tharaka Nithi area range from as low as 14ºC to 30ºC in highland areas and 22ºC to 36ºC in lowland area. Chogoria and Chuka are the fast-growing towns in Tharaka Nithi County with higher population (KNBS, 2019).

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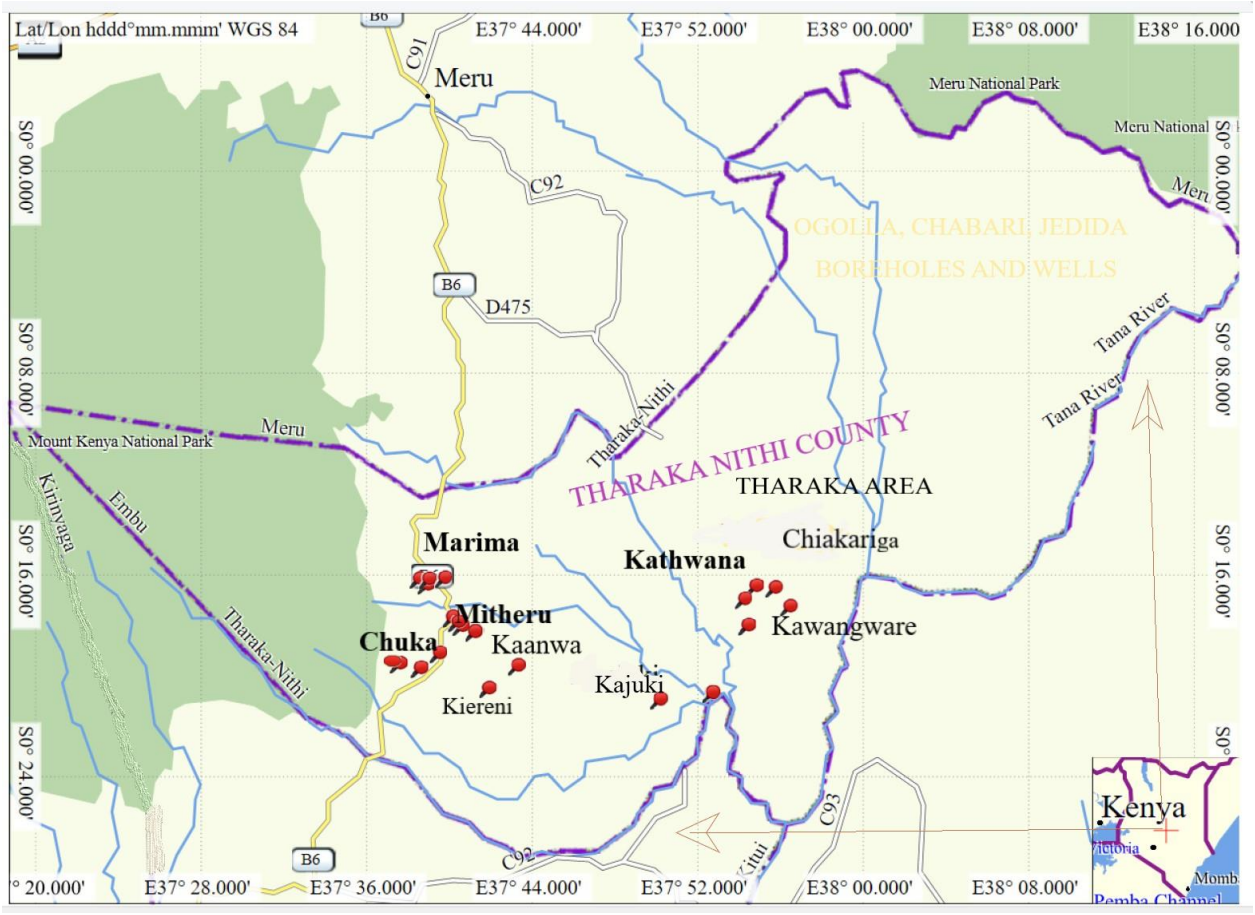

Figure 1: Map of Tharaka Nithi County Showing Locations Where Borehole Water Samples Were Collected (Red Markers)

Field Sample Collection and Laboratory Procedures

Collection of Water Samples from Respective Boreholes

One hundred and eight (108) water samples from 36 boreholes were collected from nine different locations in Tharaka Nithi County between April to September 2019 during the dry season in the area. The nine locations were Mitheru, Maarima (Maara Constituency) Chuka, Kierini, kajuki, Kaanwa (Igamba Ngombe Constituency) and Chiakariga, Kajiambao, Kawangware (Maara constituency). Four boreholes from every nine of the locations were randomly picked for sample collection. In every borehole, water samples were collected in triplicate in a 1 L plastic bottles pre sterilized with 70% ethanol. A manila rope that had been dipped in 70 % and air dried was tied to the bottle and on sterilized heavy silver coated metal and lowered to the borehole to draw the water. Once pulled out of the well, the bottles were aseptically closed, labeled, packed in the cool boxes and transported to Chuka University for analysis.

Evaluation of Total Coliform Bacteria

Most Probable Number (MPN) method was used to assess the total coliform bacteria status of water samples collected from boreholes from different locations in Tharaka Nithi County. Presumptive test was done in a 3:3:3 McCartney bottle arrangement criterion using double strength and single strength MacConkey purple broth. Durham tubes were placed inside each McCartney bottle in an inverted position. The first three sets of McCartney bottles were filled with 10 ml double strength MacConkey purple broth while second and third set of McCartney bottle were filled with 5 ml single strength MacConkey purple broth. The bottles together with their contents were sterilized in an autoclave at 121°C, 15 psi for 15 and allowed to cool in water bath at 50°C prior to inoculation with the water samples. Into the first set of 10 ml double strength MacConkey purple broth, equal amount (10 lm) of the water sample (inoculum) was added while the second and the third set of 5 ml single strength McCartney bottle were inoculated with 1 and 0.1 ml of water samples respectively. The air bubble in the Durham tubes was removed by inverting the McCartney bottle upside down gently until Durham tube was filled with the broth. Inoculated samples were incubated at 37°C for 48 hours and were examined for presence of gas in the inverted Durham and change of broth media from purple to yellow. All the tubes that had gas in the Durham tubes and the media had turned yellow were considered positive for total coliform. The probability table was used to determine the most probable number (MPN) total coliform in 100 ml of water.

Evaluation of Feacal Coliform Bacteria

Most Probable Number (MPN) method described in the preceding section was used to assess the feacal coliform bacteria status of water samples using MacConkey purple broth. A 3:3:3 McCartney bottle arrangement criterion using double strength and single strength MacConkey purple broth was used. Durham tubes were placed inside each McCartney bottle in an inverted position. The samples which turned positive for the total coliform test were used for evaluation of feacal coliform. The samples were inoculated as explained in the preceding section and were incubated at 44°C for 48 hours. At the end of incubation period, inverted Durhams were examined for gas presence and change of media from purple to yellow. All the tubes that had gas in the Durham's tubes and the media had change from purple to yellow were considered positive for feacal coliform and were used in the confirmed test. The probability table was used to determine the most probable number (MPN) total coliform in 100 ml of water. Feacal coliform positive samples were sub cultured in Nutrient Agar plates and further examined on Eosine Methylene Blue agar (EMB). Media EMB was inoculated with feacal positive bacteria isolated on nutrient agar and incubated at 37°C for 48 hours. Growth of bacteria colonies with metallic green sheen on EMB were considered as completed positive feacal coliform tests.

Total Heterotrophic Microorganism Bacteria Counts

The total heterotrophic bacteria count was done on a serially diluted water sample to disperse bacteria colonies. Water sample (1 ml) was first diluted in a 9 mL sterile distilled water as a diluent making dilution 10^1 which was subsequently diluted to dilution 10^2 and finally to 10^3. Using pour plate technique, 1ml of dilution 10^3 was plated on plate count agar in triplicate. The plates were then incubated at the temperature of 37°C for 48 hours. Individual bacteria colonies which grew were subcultured on nutrient agar and identified using biochemical test. Phenotypic traits of individual isolates (Shape, size, margin and colour) were examined

(Ogolla and Neema, 2019). Differential staining and biochemical tests which included Indole, methyl red, coagulase motility test and catalase was carried out on the isolates.

Biochemical Identification of Water Samples' Bacteria Isolates

Gram Staining of Bacteria Isolates was performed on a thin smear of pure colony prepared on a clean glass slide emulsified with a drop of normal saline. The smear was air dried and heat fix over the flame for 30 seconds. Fixed smear was flooded with crystal violet stain (primary stain) and rinsed off under slowly flowing tapwater. Gram iodine (A mordant) was poured on the smear to link the smeared cell and the crystal violate and left to stand for 1 minute before rinsing. Three drops of 80% ethanol were added to the smear and left for 1 minute to decolourise the gram-negative bacteria and rinsed was rinsed off. After decolourisation, the smear was counterstained with safranin (Secondary stain) to stain discoursed gram-negative cells. Stained smear was dried by blotting and viewed on the microscope using x100 lens and oil immersion. Cells that stained purple colour cells considered gram positive and the pinkish to reddish stained cells were considered as gram negative bacteria cells.

Catalase test was conducted on the isolates for detection of the presence or the absence of enzyme catalase. A loop-full of 48 h pure colony of individual isolate was smeared on clean glass slide. On to the bacteria loop, a drop of 3% hydrogen peroxide (H_2O_2) was added and left to stand for 30 seconds. Formation of bubbles and gas production was considered positive catalase test reaction.

Citrate utilization test was performed to test the ability of bacteria isolates of utilizing citrate as the only carbon source. Simon's citrate medium was prepared following manufacturer's instruction and agar slant prepared on test tubes. Using flame sterilized wire, pure colonies of the individual were inoculated on Simon's citrate medium slant and incubated at 37°C 48 hours. Change of colour from green to deep blue was interpreted as a positive result (Hassan, 2019). The Oxidase test was done on Whatman No. 2 filter paper onto which three drops of dimethyl p- phenylenediamine hydrochloride had been added. Procedure described by Shields and Cathcart (2016) was used in this test. Onto the dimethyl p- phenylenediamine moistened Whatman No. 2 filter paper, pure bacteria colony isolates were smeared on the paper and observed for reaction. Colony smear that formed purple colour on the filter paper within 30 seconds was considered positive for oxidase test.

Urease test was conducted on the bacteria isolates to evaluate their ability to hydrolyze urea and produce carbon dioxide and ammonia. Procedure described by Brink (2016) was used for this test. Individual pure colony isolate was inoculated on urea medium slants then incubated at 37°C and observed at the 6 hours, 24 hours, and every day for up to 6 days. The slants were observed for the development of Red-pinkish colour. The test was significant in differentiating urease-positive Proteae from other Enterobacteriaceae. Coagulase test was performed using Slide method using procedure described by Public Health England (PHE) 2018). Bacteria isolate was placed on one end of the slide and plain water that served as control to the test on the other end. The rabbit plasma was added to bacteria colony and on water on the slide respectively. The content of each slide end was emulsified using sterile applicator stick for one minute and observed for agglutination. Colonies which agglutinated on emulsification with rabbit plasma were considered positive for coagulase test. Coagulases enzymes are produced by bacteria *Staphylococcus aureus.* In this reaction, protease enzyme

converts fibrinogen to fibrin and results in visible clotting of blood (Rakotovao-Ravahatra *et al.*, 2019).

Indole test was conducted to detect indole activity on bacteria isolates. Media used for indole test was made by dissolving 5 g of sodium chloride, 10 g of tryptophan and 10 g of tryptophan in 100 ml distilled water. The broth prepared was dispensed in the test tubes then autoclaved at 121°C at 15 psi and for 15 minutes. Bacteria isolates were sub cultured in the tryptophan broth in test tubes and dried paper strips impregnated with oxalic acid inserted. Test tubes and their content were incubated for 14 days at room temperature. The strip was observed for the formation of pink colour after every two days during incubation period. The role of indole test was to determine isolates' ability to split indole from tryptophan and help differentiate Gram-negative *Bacilli* particularly the *enterobacteriaceae*.

RESULTS

Total and Feacal Coliform in Borehole Water Samples in Different Locations

The respective colony counts for total and faecal coliform in all the locations sampled were different and statistically significant ($p<0.05$). Mean total counts of colony forming units varied from one location of sampling to the next ($F(7, 36) = <.0001$; alpha=0.05). Kawangware in Kathwana recorded both high means of total coliform and feacal coliform counts of (960.8 cfu/100 ml) and (17.54 cfu/100 ml) respectively (**Table 1**). The lowest mean count of total coliform and feacal coliform (22.55 cfu/100 ml) and (5.515 cfu/100 ml) respectively was observed in boreholes at Kajiampao. Means of respective boreholes in different locations are presented (T**able 2)**.

Table 1: Comparison of Most Probable Number Means of Total and Feacal Coliforms Bacteria in Boreholes Water Samples for Different locations

Location	Total Coliform cfu/100 ml	Faecal Coliform cfu/100 ml
Kawangware	960.8^{a}	17.54^{a}
Kajuki	485.0^{b}	15.50^{b}
Chiakariga	366.33^{bc}	14.80^{b}
Mitheru	217.42^{cd}	12.80^{c}
kiereni	262.0^{d}	10.77^{d}
Kaanwa	70.0^{e}	7.83^{e}
Marima	51.58^{e}	6.99^{e}
Chuka	35.75^{e}	6.77^{e}
Kajiampao	22.55^{e}	5.52^{e}
Cv	50.906	18.53
Mean	276.879 cfu/100 ml	12.892 cfu/100 ml
LSD	115.31	0.168

Means followed by the same letter superscripted in a column are not significantly different

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Total and Feacal Coliform in Borehole Water Samples in Different Boreholes and Locations

Total coliform and feacal coliform means of boreholes in Kawangware were different and statistically significance (p<.05; Table 2). Total coliform of all boreholes in Kawangware were above 900 cfu/100 ml. Borehole number (BNo.) 1 and 4 had the highest total cfu mean of 1103.01 while BNo. 3 recorded lower mean of 903 cfu/100 ml. Feacal coliform in all the boreholes in Kawangware were above 50 cfu/100 ml with borehole (BNo.3) recording the highest feacal coliform mean of 102.76 cfu/100 ml. Only borehole (BNo. 4) recorded the lowest feacal coliform mean of 57.59 cfu/100 ml which was below the overall feacal coliform mean (74.11cfu/100 ml) recorded in Kawangware (Table 2).

Total coliform and feacal coliform means of boreholes in Kajuki were different and statistically significance (p<.05; Table 2). Higher total coliform was recorded in BNo. 3 and followed by BNo.1with mean difference of 502.27 cfu/100 ml. However, feacal coliform means difference between BNo. 3 and BNo. 1 in Kajuki were not significantly different (Table 2). The lowest total coliform was recorded in boreholes at Chuka and the mean cfu between the bore holes were different and statistically significance (p<.05; Table 2). The highest total coliform in bore holes in Chuka was 37.78 cfu/100 ml at BNo.4 while the lowest total coliform was observed at BNo.3 (13.3 cfu/100 ml). The lowest feacal coliform means were recorded in bore holes at Kajiampao (4.52 cfu/100 ml), and Chuka (4.57 cfu/100 ml) with five out of eight boreholes recording lowest feacal coliform after log transformation of the data (Table 2).

Table 2: Total and Faecal Coliform Means for Different Borehole (MPN CFU/100 ml)

Location	Borehole Number (BNo.)	Total Coliform Mean	Statistics	Feacal Coliform Mean	Statistics
Chiakariga	BNo. 1	240.005 ^{b}	Mean=209.67	33.2 ^{ab}	Mean=30.18
	BNo. 2	140.26^{c}	Lsd = 1.419	26 ^{ab}	Lsd=2.49
	BNo. 3	54.85 ^{d}	Cv = 3.479	15.14^{b}	Cv= 14.225
	BNo. 4	1031.33^{a}	F(3,12) <.0001	63.46^{a}	F(3,12) =0.039
Chuka	BNo. 1	28.46 ^{ab}	Mean= 23.464	3.0 ^{b}	Mean=4.57
	BNo. 2	21.19 ^{b}	Lsd= 1.371	6.60 ^{a}	Lsd=1.44
	BNo. 3	13.30 ^{c}	Cv= 5.304	3.0 ^{b}	Cv= 12.745
	BNo. 4	37.78 ^{a}	F(3,12)= .0004	7.34 ^{a}	F(3,12)= 0.0005
Kaanwa	BNo. 1	80.83 ^{a}	Mean=69.499	16.49 ^{a}	Mean=6.07
	BNo. 2	76.80 ^{a}	Lsd=1.421	7.27 ^{b}	Lsd=1.56
	BNo. 3	42.46 ^{b}	Cv=4.399	3.0 ^{c}	Cv= 13.009
	BNo. 4	88.84 ^{a}	F(3,12)=.005	3.78 ^{c}	F(3,12)<.0001
Kajiampao	BNo. 1	21.95 ^{b}	Mean=16.476	3.0 ^{b}	Mean=4.52
	BNo. 2	119.32 ^{a}	Lsd=2.870	12.27 ^{a}	Lsd=1.48
	BNo. 3	6.08 ^{c}	Cv=19.99	3.0 ^{b}	Cv= 13.868
	BNo. 4	4.63 ^{c}	F(3,12) =.0004	3.78 ^{b}	F(3,8)

Kajuki	BNo. 1	463 [b]	Mean=415.55	51.28 [a]	Mean=35.52
	BNo. 2	258.3 [c]	Lsd = 1.223	20.95 [c]	Lsd=1.17
	BNo. 3	965.27 [a]	Cv =1.774	49.61 [a]	Cv= 2.319
	BNo. 4	258.3 [c]	F(3,12) <.0001	29.86 [b]	F(3,12)<.0001
Kawangware	BNo. 1	1103.01 [a]	Mean=1031.82	60.36	Mean=74.11
	BNo. 2	1031.83 [a]	Lsd =1.115	84.11	Lsd=1.81
	BNo. 3	903 [b]	Cv =0.832	102.76	Cv= 7.325
	BNo. 4	1103.01 [a]	F(3,12) = .0086	57.59	F(3,12)= 0.1531
Marima	BNo. 1	18.17	Mean=33.06	4.16	Mean=5
	BNo. 2	53.89	Lsd=6.44	6.0	Lsd=2.03
	BNo. 3	21.43	Cv=28.278	3.98	Cv=23.4
	BNo. 4	56.94	F(3,12) =.4022	6.32	F(3,12)=0.362
Mitheru	BNo. 1	54.85 [bc]	Mean=94.38	17.06	Mean=19.07
	BNo. 2	145.92 [ab]	Lsd= 3.61	30.36	Lsd=3.33
	BNo. 3	451.65 [a]	Cv=14.98	25.81	Cv = 21.67
	BNo. 4	21.95 [c]	F(3,12) .0034	9.89	F(3,12) = 0.218
Kiereni	BNo. 1	903 [a]	Mean=96.81	63.622[a]	Mean=11.95
	BNo. 2	73 [b]	Lsd =1.39	11.92[b]	Lsd=1.94
	BNo. 3	59.55 [b]	Cv= 3.83	8.96[b]	Cv= 14.21
	BNo. 4	22.37 [c]	F(3,12) <.0001	3.0[c]	F(3,12)<.0001

Means followed by the same letter superscripted in a column in every category are not significantly different

Bacteria Contaminants of Borehole Water Samples

Bacteria Species Contaminants of Borehole Water Samples

The bacteria species contaminants in boreholes in Tharaka Nithi County differed significantly (F (7, 36) = <.0001; alpha =0.05). *Escherichia coli* (*E. coli*) were the mostly isolated bacteria contaminant with mean of 15.29 cfu followed by *Bacillus spp* with mean of 8.02 cfu. The least isolated bacteria contaminant was *Pseudomonas spp* with mean of 5.11 cfu. Differences between *E. coli* and *Bacillus spp, Klebsiela spp, Proteus spp, Streptococcus spp, Enterobactor spp* and *Psuedomonas spp* were 0.280, 0.297, 0.353, 0.359, 0.437 and 0.476 cfu in the order listed and were statistically significance (Table 4).

Table 3: Bacteria Species Contaminants of Boreholes

Bacteria Isolate	**Means CFU**
E. coli	15.29 [a]
Bacillus spp	8.02 [b]
Klebsiellaspp	7.72 [bc]
Proteus spp	6.78 [c]
Streptococcus spp	6.69 [c]
Enterobacter spp.	5.59 [d]
Psuedomonasspp	5.11 [d]
Mean	7.4085
CV	18.694
LSD	0.0435

Means followed by the same letter superscripted in a column are not significantly different

Bacteria contaminants varied from one location of sampling to the next. Kawangware recorded the highest number of all bacteria contaminants except *Klebsiela spp.*

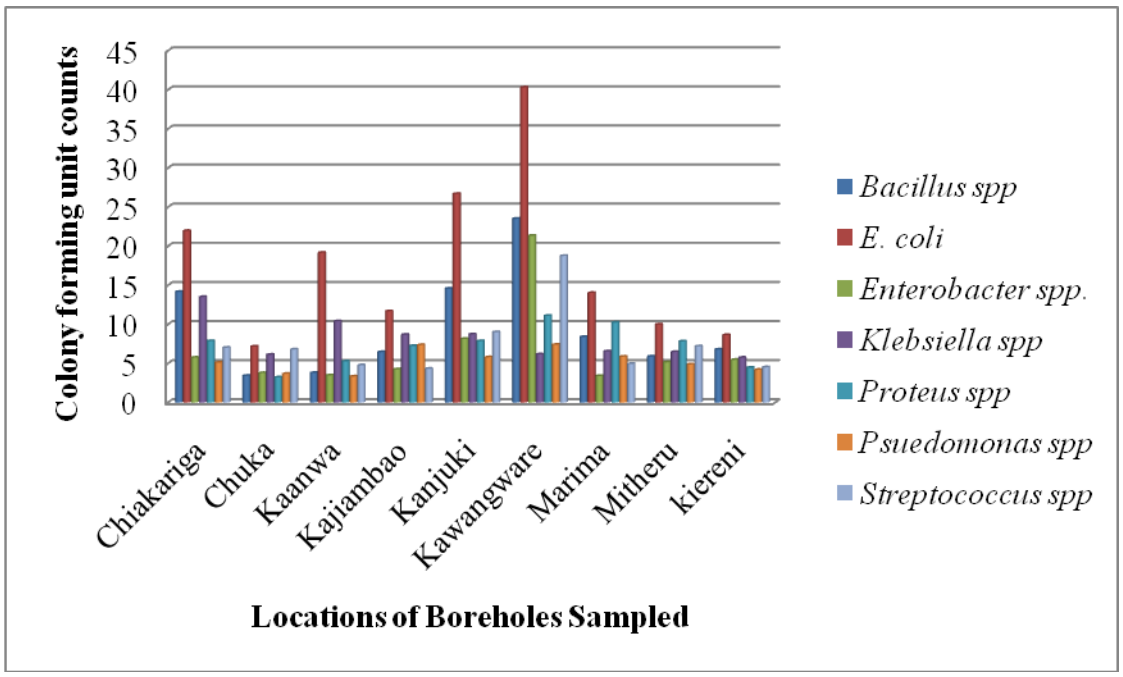

Figure 2: Graph of bacteria species contaminants in borehole water in Tharaka Nithi County

Phenotypic and Biochemical Traits of Bacteria Water Contaminants

Phenotypically, bacteria isolated from water samples were different in terms of colour, shape, pattern of growth margin and size. Whereas isolate 1 was small in size, creamy in appearance and had rough surface, others such as isolate 2 was medium sized and was greenish in colour (Table 3). Equally, biochemical tests carried on the isolates demonstrated that they were different and distinct. Whereas the coagulase test was negative for all the isolates, the results of other biochemical test conducted were varied (Table 3).

Bacteria Species Water Contaminants for Individual Boreholes

In Kawangware, *E. coli* had mean count of 40.31 cfu followed by *Bacillus Spp* (23.51) and *Klebsiela spp* was the least with mean count of 6.17 cfu. Boreholes in Chuka recorded the lowest mean of *Bacillus* spp (3.43±1.23 cfu), *E. coli* (7.16±1.31) and *Klebsiella spp* (6.12±1.42). Marima recorded the lowest mean count of *Enterobacter spp.* (3.40±1.42 cfu). However, *Klebsiella spp* highest mean (13.50±1.33 cfu) was recorded in Chiakariga. Detailed means and standard deviations of respective boreholes in different locations and respective bacteria species contaminants are presented in **table 5**.

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Table 4: Locations, Boreholes and their Respective Bacteria species Contaminants

Location		*Bacillus spp*	*E. coli*	*Enterobacter spp.*	*Klebsiella spp*	*Proteus spp*	*Psuedomonas spp*	*Streptococcus spp*
Chiakariga	Borehole 1	19.85± 1.16	22.10± 1.20	5.52 ±1.34	15.92 ±1.13	10.98 ±1.1	3.92 ±1.3	10.26 ±1.16
	Borehole 2	15.92 ±1.13	22.15 ±1.17	7.23 ±1.23	14.49 ±1.21	9.16 ±1.26	4.48 ±1.43	11.32 ±1.05
	Borehole 3	4.93 ±1.23	13.84 ±1.20	3.30 ±1.18	9.16±1.26	3.56 ±1.34	4.22 ±1.34	3.0±1
	Borehole 4	25.79 ±1.17	34.32±1.04	8.28 ±1.16	15.74 ±1.25	10.72 ±1.32	9.16±1.26	6.95 ±1.73
Chuka	Borehole 1	3.0 ±1	6.46 ±1.35	3.63 ±1.18	5.65 ±1.11	3.30 ±1.18	3.30 ±1.18	8.24±1.20
	Borehole 2	3.92 ±1.29	7.23 ±1.23	3.0 ±1	7.61 ±1.48	3.63 ±1.18	3.30 ±1.18	4.22±1.343
	Borehole 3	3.92 ±1.29	7.11 ±1.59	3.0±1	7.06 ±1.68	3.0 ±1	4.0 ±1	7.34±1.40
	Borehole 4	3.0 ±1	7.88 ±1.23	6.26±1.21	4.64±1.14	3.0 ±1	4.22 ±1.343	8.25 ±1.197
Kaanwa	Borehole 1	3.0 ± 1	13.08±1.74	3.30 ±1.18	14.70 ±1.29	6.80 ±1.34	3.302 ±1.181	11.50 ±1.24
	Borehole 2	3.0 ±1	18.84 ±1.17	3.78 ±1.49	15.223±1.156	7.65 ±1.08	4.16 ±1.42	4.217 ±1.34
	Borehole 3	4.22 ±1.34	29.45 ±27	3.0 ± 1	6.952 ±1.732	3.557±1.343	3.0 ± 1	3.0 ± 1
	Borehole 4	5.65 ±1.11	18.61 ±1.29	3.915 ±292	7.560 ±768	4.160±1.417	3.0 ±1	3.56 ±1.34
Kajiampao	Borehole 1	9.52 ±1.49	18.87 ±1.16	3.78 ±1.49	11.6 ±1.14	13.10 ±2.34	8.28 ±1.16	5.74 ±1.78
	Borehole 2	12.27 ±1.13	23.30 ±1.07	7.37±1.43	16.85 ± 1.34	12.76 ±1.27	9.89±1.20	4.38±1.54
	Borehole 3	4.93±1.63	5.59±1.21	3.0±1	5.518±1.34	3.0±1	4.38±1.54	3.0±1
	Borehole 4	3.0±1	7.56±1.23	3.92±1.29	5.24±1.6	5.49±1.92	8.14±1.56	4.642±1.137
Kajuki	Borehole 1	17.54±16	24.25±1.84	4.58 ±1.66	9.73±2.19	12.576±1.38	5.13±1.73	7.06±1.68
	Borehole 2	11.70±1.61	13.37±1.70	8.85±1.25	9.76±1.78	9.17±1.3	7.49±2.50	6.75±1.66
	Borehole 3	22.65±1.05	43.24±1.08	10.25±1.42	15.38±2.00	11.04±1.34	9.740±1.336	13.658±1.044
	Borehole 4	9.70±1.82	36.18±12	10.59±1.73	3.98±1.31	3.0±1	3.0±1	10.13±2.30
Kawangware	Borehole 1	25.56±1.36	44.29±1.06	18.72±1.24	4.380±1.54	9.03±2.12	6.60±3.92	21.34±1.24
	Borehole 2	23.96±1.07	31.62±1.07	25.84±1.15	12.63±1.09	16.94±1.11	11.89±1.18	19.34±1.06
	Borehole 3	19.51±1.65	43.60±1.07	23.61±1.09	8.73±2.00	14.52±1.20	10.77±1.30	20.33±1.25
	Borehole 4	25.56±1.12	43.24±1.08	18.17±1.18	3.0±1	6.84±1.52	3.56±1.34	14.8±1.23
Marima	Borehole 1	5.52±2.27	3.30±1.18	4.48±2.00	3.56±1.34	4.93±1.23	4.76±2.23	3.63±1.18
	Borehole 2	11.94±3.33	14.64±1.32	3.0±1	3.56±1.34	15.47±1.98	8.29±2.06	3.63±1.18
	Borehole 3	19.35±1.25	23.32±1.24	3.0±1	4.48±1.43	13.03±1.30	8.43±1.34	5.13±1.73
	Borehole 4	3.92±1.29	34.40±1.12	3.30±1.18	32.23±1.1	11.05±1.10	3.56±1.34	8.85±1.25

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Location		*Bacillus spp*	*E. coli*	*Enterobacter spp.*	*Klebsiella spp*	*Proteus spp*	*Psuedomonas spp*	*Streptococcus spp*
Mitheru	Borehole 1	6.54±1.59	7.65±1.87	4.93±1.63	8.65±1.42	6.6±2.04	4.22±1.34	8.04±1.86
	Borehole 2	5.59±1.24	11.79±1.27	6.14±1.93	5.24±1.66	10.54±1.63	3.63±1.18	6.3±1.49
	Borehole 3	10.95±3.08	17.62±1.28	6.46±2.52	6.84±1.61	18.0±2.62	12.31±3.40	17.54±1.44
	Borehole 4	3.0±1	6.26±1.21	3.56±1.34	5.65±1.52	3.0±1	3.0±1	3.0±1
kiereni	Borehole 1	4.72±1.53	7.88±1.23	4.58±1.26	10.63±1.11	7.61±1.16	6.46±1.35	4.31±1.14
	Borehole 2	3.979±63	6.07±1.44	4.16±1.42	3.56±1.34	5.24±1.60	4.22±1.34	4.48±1.43
	Borehole 3	11.55±1.19	11.75±1.29	7.96±1.13	6.26±1.21	3.30±1.18	3.78±1.49	6.54±1.27
	Borehole 4	9.78±1.29	9.87±1.23	5.85±1.31	4.48±1.43	3.0±1	3.0±1	3.30±1.18

Table 5: Phenotypic and Biochemical Traits of Bacteria Isolated from Borehole Water

Isolates	Colony charateristics	Gram stain	Catalase	Oxidase	Coagulase	Indole	Citrate	Ureas	VP	Species
1	Small Creamy, rough surface	+ve rod chain	-ve	-ve	-ve	-ve	+ve	+ve		*Bacillus spp*
2	Medium greenish, entire margin, low convex	-ve rod single	+ve	+ve	-ve	-ve	+ve	-ve	+ve	*Psuedomonas spp*
3	large Creamy, rough surface	-ve rod single	+ve	-ve	-ve	-ve	+ve	+ve	+ve	*Klebsiella spp*
4	Medium cream, convex, smooth margin	-ve rod single	+ve	-ve	-ve	+ve	-ve	-ve	+ve	*E. coli*
5	Cremi, circular, shiny	cocci	+ve	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus spp.*
6	Small, white, circular, dome shaped	-Rod shaped	+ve	-ve	-ve	+ve	-ve	+ve	-ve	*Proteus spp*
7	Small, whitish, water colonies	-ve rod single	+ve	-ve	-ve	-ve	+ve	+ve	+ve	*Enterobacter spp.*

DISCUSSION

Total and Feacal Coliform Contamination of Borehole Water

Higher total coliform count that exceeds the World Health Organization (WHO) international standards (<1 coliform/100 ml) was observed in the entire borehole sampled across Tharaka Nithi County. Our results are in line to those of Adogo *et al*. (2016) and Ayika *et al.* (2019). Though used as an indicator of water quality, high total coliform count may however not necessarily point at fecal contamination. According to Bartram *et al.* (1996), total coliforms result from environmental and/or thermo tolerant (fecal) coliforms due to entry of organic matter or soil in water. The higher levels of total coliform observed may be attributed to elevated temperatures experienced in Tharaka Nithi County. As pointed out by Bello *et al.* (2013), microorganisms proliferation may be affected by variation of prevailing temperatures. Positioning and distance of pit latrines dug a few meters from the boreholes may be the source of total coliform entry in boreholes through underground leaching or seepage from bio solids especially for shallow boreholes (Moyo, 2013; Seth *et al.*, 2014). Coliform count varied from one borehole to the next and from region to region. Variation in coliform count of different borehole may be due to variation in soil type, rocks and surface through which water flow as explained by Palamuleni and Akoth (2015), Obioma *et al*. (2018) and Ayika *et al*. (2019). Soils around Kathwana, Kajiampao, Chiakariga and Kawangware are sandy and/or rocky and may allow microorganisms to sip into the well and boreholes.

During sample collection, it was observed that an activity such as washing of utensils, clothes and feeding of animals were done just on/or a few meters from the boreholes which may also explain the high count of bacteria observed. Most of the boreholes use hand pulled ropes to draw water from the wells especially in the locations such as Maara, Mitheru and Chuka. Pulling of water from the wells may cause water spillage that flow back to the well, thus, contamination. External contaminants such as buckets tied that are used to pull water from the boreholes may as well have contributed for borehole contamination. Faecal coliform colony forming unit count varied from borehole to borehole and from location to location and the differences were statistically significant ($p<.05$). Our finding on higher contamination of borehole water with feacal coliform corroborates to those of Orotho and Fweji (2012), Adogo*et al*. (2016) and Ayika *et al.*(2019). However, our findings differ with those of Oria-Usifo *et al.* (2018) which reported no feacal coliform in borehole water. Reasons for contamination of boreholes water with feacal coliforms may be the same as for total colifoms stated above.

Bacteria Contaminants of Borehole Water

Bacteria isolates from different locations and for individual boreholes were statistically significant ($p < 0.05$). Bacteria such as *Klebsiella spp* and *E. coli* which are thermo tolerant coliforms were isolated in boreholes water sample from different locations. Presence of such bacteria in water samples may point at possible faecal contamination of boreholes with human or animal wastes. *Klebsiella* spp inhabits varied environments and has the ability to multiply in water surface with high nutrients (WHO, 2017). According to Ainsworth (2004), *Klebsiella* incidence in water and subsequent ingestion may however not result in gastro

intestinal illness. Nonetheless, occurrence of *Klebsiella* spp in borehole water sample in Tharaka Nithi County should be treated with a lot of concern due to its medical significance. Presence of *E. coli* is a health concern and may lead to diseases both in children and adult (WHO, 2017).

Suspected *Proteus* spp an *enterobacteriaceae* was isolated from different borehole water samples in this study. *Proteus* spp may have originated from either natural habitat of human waste which may be are its host. According to Drzewiecka (2016), *Proteus* spp is a known human opportunistic pathogen and may be found in human's gastro intestinal tract particularly in the intestines Consumption of water contaminated by *Proteus* spp may cause nosocomial infections in infants, children and immune compromised individual (O'Hara *et al.,* 2000). Our study finding on bacteria species contaminants corroborates to other findings in different studies of boreholes and wells (Ortiz, 2007; Palamuleni and Akot., 2015; Adogo *et al.,* 2016)

Future Study

In order to create a comprehensive understanding on bacterial contamination of boreholes and wells in Tharaka Nithi County, study of the borehole's bacteria contaminants throughout the year and on monthly basis is necessary. This will generate data on monthly and seasonal bacterial borehole water quality in the county.

CONCLUSION

1. Boreholes in TharakaNithi County have total coliform and feacal coliform counts beyond the WHO recommendation of 0 cfu / 100 ml of water.
2. Borehole contamination in Tharaka Nithi County varies from location to location and from borehole to borehole.
3. Bacteria species that may be of health concern exists in boreholes in Tharaka Nithi County and vary from one borehole to the next and from location to location.
4. Boreholes around Kawangware in Tharaka region have higher total and feacal count compared to other locations sampled.

RECOMMENDATIONS

There is need to educate households in Tharaka Nithi County who depend on borehole water for domestic use on health concerns and the need to properly treat water prior to use since most people uses borehole water without any treatment affordable treatment methods such as boiling or chlorination. There is need to educate community on position and safe location of latrines relative to borehole location. Where provision of tap water is not possible, effort should be made by the county government to refurbish deteriorated boreholes. Regular monitoring of boreholes

is recommended that may create awareness on the statues of borehole water quality hence avoidance of disease outbreak.

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