Chuka University
info@chuka.ac.ke | 020 231 0512 / 020 231 0518

BACTERIOLOGICAL QUALITY ASSESSMENT OF DOMESTIC BOREHOLES IN THARAKA NITHI COUNTY, KENYA

KithakaSamsonChabari,[1*]FredrickO.Ogolla,[2]andJedidaKarimi[1]
[1]ChukaUniversity,DepartmentofEnvironmentalStudiesandResourcesDevelopment,P.O.Box109-60400,Chuka Email: Kithakasam@yahoo.com, Email: kariukijd@yahoo.com
[2]ChukaUniversities,DepartmentofBiologicalSciences,P.O.Box109-60400,Chuka Email: Ogolla.fredy@gmail.com
jnchabari@chuka.ac.ke; fogolla@chuka.ac.ke; jkkarimi@chuka.ac.ke

How to cite:

Chabari, K. S., Ogolla, F. O. and Karimi, J. *(*2021). Bacteriological Quality Assessment of Domestic Boreholes in Tharaka Nithi County, Kenya. *In: Isutsa, D. K. (Ed.). Proceedings of the 7th International Research Conference held in Chuka University from 3rd to 4th December 2020, Chuka, Kenya,* p.59-78

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 contaminationbypathogenic microorganismsfrom nearbytoilets,defecationfromdomesticatedanimalsand surface runoff.Despiteofknown healthconcernsofconsumingcontaminated water manyruralresidentsconsumeborehole water whose portability is not determined and may not meet WHO standards. Thus, this study was conducted in Tharaka Nithi County to determine bacterial safety of selected wells and boreholes in Maara, IgambaNgombe and Tharaka Constituencies between March to September 2019. A total of 108 water samples were collected from 36 boreholes. 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 different locations of study and boreholes were 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 in tharakasouth.Coliformbacteria*E.coli,Bacillusspp,Klebsiellaspp*wereisolated inallthewatersampled.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

INTRODUCTION

InKenya, expendituretowards water projectsbytheNationalgovernmentinsomecountieshasincreased.However, long droughts, high poverty, cost of access to piped water, insufficient planning and managing limit access to water (Development Initiatives, 2018). Shortage of water has seen increased use of wells and boreholes as alternativewater source for drinking by human and livestock, irrigation and even for industrial processes(Onuorah *et al.*, 2019). However, water contamination is a significant challenge when it comes to water quality. Contamination has made provision of clean, safe and portable water a challenging event in most developing countries in Africa Kenya included (Dara and Mishra, 2011; Idibie*et al*., 2018). According to Water Resource Authority ([WRA], 2018), in Kenya shortage of portable water may worsen by the year 2030 due to degradation of its quality by human activity. Ground water contamination may arise from runoff or flood splash, leachate from buried waste, latrine and septic tanks into the borehole (Obot and Edi, 2012; Sebiawu*et al.*, 2014; Sila, 2019). Most untreated water sources are contaminated with faeces, animal and plant wastes (Idibie*et al*., 2018). Despite of eminent health concern about 1.5 billion global populations depend on ground water (Palamuleni and Akoth, 2015). Majority rural residents consume water whose portability is not determined and may not meet WHO standards (Obioma *et al.*, 2017). Water degradation may results from bacterial, fungal, and parasites, physical and dissolved chemicals(Babič *et al.*, 2017).

Microbial contaminants of drinking water are bacteria generally termed coliforms represented by *E. coli*. O*ther microorganisms are Giardia lamblia and Cryptosporidium parvum*(Opara and Nnodim, 2014). Indicator organisms

such as *Klebsiella,Escherichia coli* and*Enterobacterbacteria* species in water may be an evidence of existence pathogens of *Salmonella, Clostridium pafringens*and Protozoa (Anyamene and Ojiagu, 2014). Illness such as diarrhea, giardiasis, dysentery, and gastroenteritis are implication of consumption of contaminated water (Isikwue and Chikezie, 2014; Oludairo and Aiyedun, 2016). Infection by these bacteria is symptomized by stomach cramps, nausea, vomiting and fevers occurring between two to ten days upon consumption in water (Ugwuzor and Ifeanyi, 2015). Fungal contaminants in borehole water may include Aspergillus, Penicillium Rhizopus and Fusarium (Okpako*et al.,* 2009). Other fungal pathogen which have been isolated from drinking water include *Alternariaspp, Exophialaspp, Candidaspp, Acremoniumspp, Mucorspp,Absidiaspp, Rhodotorulaspp*(Arvanitidou*et al.* 1999, Gunhild*et al.*,2006; Ayanbimpe*et al.,* 2012) (Onuorah *et al.,* 2019).

Health implication of fungal water contaminated causes allergenice reaction or infections in people whose immune system is compromised individuals (Gunhild*et al.,* 2006; Warris, 2003; Ayanbimpe*et al.,* 2012). Respiratory problems, mucosal, subcutaneous rhinocerebral, and cutaneous infections are other medical issues involving fungal water contamination (Hoog *et al.,* 2000).Populations group prone to fungal infections, those pregnant, those with diabetes mellitus, and those withimpairments incirculatorysystem. Other categories of with higher susceptibilityto fungal infection include persons with chronic pulmonary obstructive disease, cancer, HIV and cystic fibrosis (Doggett, 2000; Babič *et al.,* 2017). Despite medical significance fungal role in water contamination has been given little attention particularly for borehole water sources thus, poorly understood (DEFRA), 2011). Research istherefore necessary to explore the fungal contaminants in community boreholes and protect vulnerable population from impending infection.

METHODOLOGY

Preparationofculturemedia

Potato dextrose agar, Nutrient agar, MacConkey purple broth culture media were used to for the study. Potato dextrose agar was used to isolate fungal contaminants. Nutrient agar was used for total bacteria count. MacConkey purple broth was used for total coliform and feacal coliform evaluation. Methylene blue agar was used to confirm presence of feacal coliform after positive presumptive test in MacConkey agar. Nutrient agar was prepared by dissolving 28 g of nutrient agar (NA) powder in 1 liter distilled sterile water in a conical flask. The media was dissolved by heating on flame while stirring then covered with cotton wool and autoclaved at 121°C,15 psi for 15 minutes and cooled to 50°C in water bath. Fifteen (15) ml, of the cooled media was dispensed sterile Petri dishes aseptically.Potato dextrose agar was prepared by dissolving Thirty-nine grams of potato dextrose agar powder was dissolved in distilled water (1L)by heating. The media was covered using cotton wool and autoclaving at 121°C, 15 psi for 15 minutes then cooled to 50°C.Cooled media was supplemented with 50 mg of antibiotic and 15 ml dispensed sterile Petri dishes aseptically.

EvaluationofTotalColiformBacteria

Most Probable Number (MPN) method described was used to assess the total coliform bacteria status of water samples. Presumptive test was done in a 3:3:3 McCartney bottle arrangement criterion using double strength and single strengthMacConkeypurple broth. Durhamtubes were placed inside the eachMcCartneybottle inaninverted position. The first three sets of McCartney bottles was filled with 10ml double strength MacConkey purple broth while second and third set ofMcCartney bottle 5 ml single strength MacConkeypurple broth. The bottles together with their contents were sterilized in an autoclave at 121°C, 15 psi for 15 and allowed to cool prior to inoculation with the water samples. Into the first set of double strength MacConkey purple broth, equal amount (10 lm) of the sample(inoculum) wasaddedwhilethesecondandthethirdsetofsinglestrengthMcCartneybottle wereinoculated 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 in it was filled with the media. Inoculated samples were incubated at37°C for 48 hoursand wereexamined gasproductionintheinverted durham and changeof mediafrom purple to yellow. All the tubes that had gas in the durham tubes and the media had change from purple to 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.

EvaluationofFeacalColiformBacteria

MostProbableNumber(MPN) method was usedtoassesstotalcoliformbacteria in water samples.Presumptivetest was done in a 3:3:3 McCartney bottle arrangent criterion using double strength and single strength MacConkey purplebroth.Durhamtubes wereplacedinsidetheeachMcCartneybottleinaninvertedposition.The firstthree sets of McCartney bottles was filled with 10 ml double strength MacConkey purple broth while second and third set of McCartney bottle 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 prior to inoculation with the water samples.Into the first set of double strength MacConkey purple broth, equal amount (10 lm) of the sample (inoculum) was added while the second and the third set of single strength McCartney bottle were inoculated with 1 and 0.1 ml of water samples respectively. The air bubble in the Durham's tubes was removed by inverting the McCartney bottle upside down gently

until Durham's tube in it was filled with the media. Inoculated samples were incubated at 44°C for 48 hours and were examined gas production in the inverted durhamand change of media frompurple to yellow.

All the tubes that had gas in the Durham's tubes and the media had change from purple to yellow were considered positivefortotalcoliformandwereusedintheconfirmedtest.Aprobabilitytablewasusedtodeterminethemost probable number (MPN) total coliform in 100 ml of water. Eosine Methylene Blue agar was used to confirm the positive feacal coliform positive results. Using flame Eosine Methylene Blue agar (EMB) plates and followed by incubation at 37°C for 48 hours. Growth of metallic green sheenbacteria colonies on an EMB was considered to be positive test for feacalcoliform.Feacal coliform positive samples were sub cultured in Nutrient Agar and used for differential staining and biochemical tests which included Indole, methyl red, coagulase motility test and catalase.

TotalHeterotrophicMicroorganismBacteriaCounts

Thetotalheterotrophicbacteriacount wasdoneonaten-fold serialdiluted sampleto dispersebacteriacolonies.One ml of water sample was first diluted in a 9 mL sterile distilled water as a diluent making dilution 10^1 which was subsequentlydiluted to dilution 10^2 and finallyto 10^3. Using pour platetechnique, 1ml of dilution $10^3$was plated on plate count agar (CM0003, Oxoid UK) in triplicate. The plates were then incubated at the temperature of 37°C for48 hours. Individual bacteria colonies which grew were subcultured on nutrient agar and their characteristics and identity evaluated using biochemical test. Colony morphology of each isolate such as e.g. shape, size, margin, consistency, elevation and colour was examined.

GramStainingofBacteriaIsolates:

A thin smear of pure colon was made on the clean glass slide with a drop of normal saline. The smear was air dried heat fixover the flame for 30seconds. Fixed smear was flooded withcrystal violet stain(a primarystain) and rinsed using washbottle. Gramiodine (A mordant) was poured on the smear to linkthe smeared cell and the crystal violate and left to stand for 1 minute before rinsing. Three drops of 80% ethanol was added to the smear and left for 1 minute to decolourisethe gram negative bacteria and rinsed. After decolourisation, the smear was counterstainedwith 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 being gram positive and the pinkish to reddish stained cells were considered as gram negative bacteria cells (Ogolla and Neema, 2019). 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 drops 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'sinstruction and 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 colour was interpreted as a positive result. Sugar fermentation test was performed on individual isolates to test their ability to ferment sugars and produce gas and acid or only acid(Public Health England (PHE), 2019). The media made of peptone water and addition ofphenol red as an indicator was dispensed in test tubes that had inverted Durham's tubes and by autoclaved at 121°C for 15 minutes for sterility. Sugar solution (1%) was separately prepared and sterilized at 115°C for 10 minutes and5 ml dispensed in tubes that had peptone water and an indicator. Tubes were inoculated using 24 hours old bacteria isolates then incubated at 37°C. The incubated tubes were observed for acid only or gas and acid production at the end of 24 hours incubation period. Change of media from light green colour to yellow colour indicated acid production. Presence of gas in the Durham's tube wasanindicationof gas productionbythe bacteria isolates(Gram negative bacteria) that utilize different sugars (Carbon and energy source). During sugar fermentation, sufficientacid that is produced may lower the pH of the test medium causing colour change (Hassan, 2019).

The production of the acid lowers the pH of the test medium, which is detected by the color change of the pH indicator.Color change only occurs when sufficient amount ofacid is produced, as bacteria may utilize the peptone producing alkaline by products. The production of the acid lowers the pH of the test medium, which is detected by the color change of the pH indicator.Color changeonlyoccurswhensufficientamount of acidis produced, as bacteria may utilize the peptone producing alkaline by products The production of the acid lower the pH of the test medium, which is detected by the color changeofthe pHindicator.Color change only occurs when sufficient amount of acid is produced, as bacteria may utilize the peptone producing alkaline by products. Oxidase test was done on Whatman No. 2 filter paper on 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 smearedon the paper and observed for reaction. Colony smear that formed purple colour on the filter paper within 30 seconds was considered positivefor oxidasetest.Urease test wasconducted onthebacteria isolates to evaluatetheir abilityto hydrolyzeureaand 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 slant were observed for the development of Red-pinkishcolourthatindicate the presence of Urease cultures that differentiates urease-positive Proteeae from otherEnterobacteriaceae.

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. Then rabbit plasma was added to bacteria colony and on water on the slide respectively. The content of each slide end were emulsified (mixed) 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 indicating presence of coagulase enzymes. 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 oftryptophan 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 significance of indole test is to determine isolates' ability to split indole from tryptophan. Indole test differentiates Gram-negative *Bacilli* particularly the *enterobacteriaceae*.

RESULTS

TotalandFeacalcoliforminBoreholewatersamplesinTharakaNithiCounty

Total and faeacal coliform differed significantly ($p<0.05$). Mean total counts of colonyforming units varied from one location of sampling to the next ($F(7, 36)=<.0001$; alpha=0.05). Kawangware and Kathwana recorded bothhigh means of total coliform and feacal coliform counts of (960.8) and (17.54) respectively (**Table 1**). The lowest mean count of Total coliform and feacal coliform (22.55) and (5.515) respectively was observed in boreholes at Kajiampao. Means and standard deviations of respective boreholes in different locations are presented in **table 2**.

Table 5: Comparison of Most Probable Number of Total and Feacal Coliforms Bacteria in boreholes in Sampling Areas of Tharaka-Nithi County

Location	TotalColiformcfu/100ml	FaecalColiformcfu/100ml
Kawangware	960.8^{a}	17.5436^{a}
Kajuki	485.0^{b}	15.5049^{b}
Chiakariga	366.33^{bc}	14.7967^{b}
Mitheru	217.42^{cd}	12.8037^{c}
kiereni	262.0^{d}	10.7737^{d}
Kaanwa	70.0^{e}	7.8328^{e}
Marima	51.58^{e}	6.9938^{e}
Chuka	35.75^{e}	6.7661^{e}
Kajiampao	22.55^{e}	5.515^{e}
CV	50.906	18.53
Mean	276.879	12.8917
LSD	115.31	0.1684

Meansfollowedbythesamelettersuperscriptedinacolumnarenotsignificantlydifferent

BacteriaSpeciesContaminantsofBoreholeWaterSamplesinTharakaNithiCounty

The occurrence of different bacteria species in boreholes in TharakaNithi County were significantly different ($F(7, 36)=<.0001$;alpha=0.05). *Escherichiacoli*(*E.coli*)werethe mostlyisolatedbacteriacontaminant withmeancolony count of 15.29 followed byBacillus with mean of colony count of 8.02. The least isolated bacteria contaminant was *Pseudomonasspp*with mean colony count of 5.11 CFU. Differences between *E. coli* and *Bacillus spp, Klebsielaspp, Proteusspp,Streptococcusspp,Enterobactorspp*and*Psuedomonasspp*were0.280,0.297,0.353,0.359,0.437and 0.476countsintheorderlistedwerestatisticallysignificance(Table 4).

Table6:TheMPNmeansandStandardDeviationsofTotalcoliformandfeacalcoliformfordifferent borehole in Tharaka Nithi County (CFU)

Location	BoreholeNo	TotalColiformMean	feacalColiformMean
Chiakariga	BoreholeNo.1	238.33±31.75	33.20±2.41
	BoreholeNo.2	140±17.32	26.0±1
	BoreholeNo.3	53.67±18.48	15.14±1.22
	BoreholeNo.4	1033.33±115.47	63.46±1.43
Chuka	BoreholeNo.1	28±0.0	3.0±1
	BoreholeNo.2	18.67±5.69	6.60±1.18
	BoreholeNo.3	10.33.1.15	3.0±1
	BoreholeNo.4	35±0.5	7.34±1.42
Kaanwa	BoreholeNo.1	78.33±14.43	16.49±1.20
	BoreholeNo.2	75±17.32	7.27±1.19
	BoreholeNo.3	40±8.66	3.0±1
	BoreholeNo.4	86.67±14.43	3.78±1.49
Kajiampao	BoreholeNo.1	19±1.73	3.0±1
	BoreholeNo.2	116.67±11.54	12.27±1.13
	BoreholeNo.3	4.67±6.43	3.0±1
	BoreholeNo.4	2.67±4.62	3.78±1.49
Kajuki	BoreholeNo.1	460±0.0	51.28±1.06
	BoreholeNo.2	256.67±31.75	20.95±1.08
	BoreholeNo.3	966.67±115.47	49.61±1.06
	BoreholeNo.4	256.67±31.75	29.86±1.13
Kawangware	BoreholeNo.1	830.25±539.5	28.50±4.79
	BoreholeNo.2	1100±0.0	90.82±1.36
	BoreholeNo.3	900±0.0	102.76±1.09
	BoreholeNo.4	1100±0.0	57.59±1.15
Marima	BoreholeNo.1	15.33±2.89	4.16±1.79
	BoreholeNo.2	98±124.01	6.0±1
	BoreholeNo.3	31.33±38.21	3.98±1.63
	BoreholeNo.4	61.67±4193	6.32±1.09
Mitheru	BoreholeNo.1	53.67±18.48	17.06±2.77
	BoreholeNo.2	160.33±99.65	30.36±1.83
	BoreholeNo.3	636.67±456	25.81±1.56
	BoreholeNo.4	19±1.73	9.89±1.2
kiereni	BoreholeNo.1	900±0.0	63.622±1.66
	BoreholeNo.2	70±0.0	11.92±1.15
	BoreholeNo.3	58.33±18.93	8.96±1.60
	BoreholeNo.4	19.67±4.62	3.0±1

Table7:BacteriaSpeciesContaminantsofBoreholesandwellsinTharakaNithiCounty

BacteriaIsolate	Means CFU
E.coli	15.29[a]
Bacillusspp	8.02 [b]
Klebsiellaspp	7.72 [bc]
Proteusspp	6.78 [c]
Streptococcusspp	6.69 [c]
Enterobacterspp.	5.59 [d]
Psuedomonasspp	5.11 [d]
Mean	7.4085
CV	18.694
LSD	0.0435

Meansfollowedbythesamelettersuperscriptedinacolumnarenotsignificantlydifferent

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

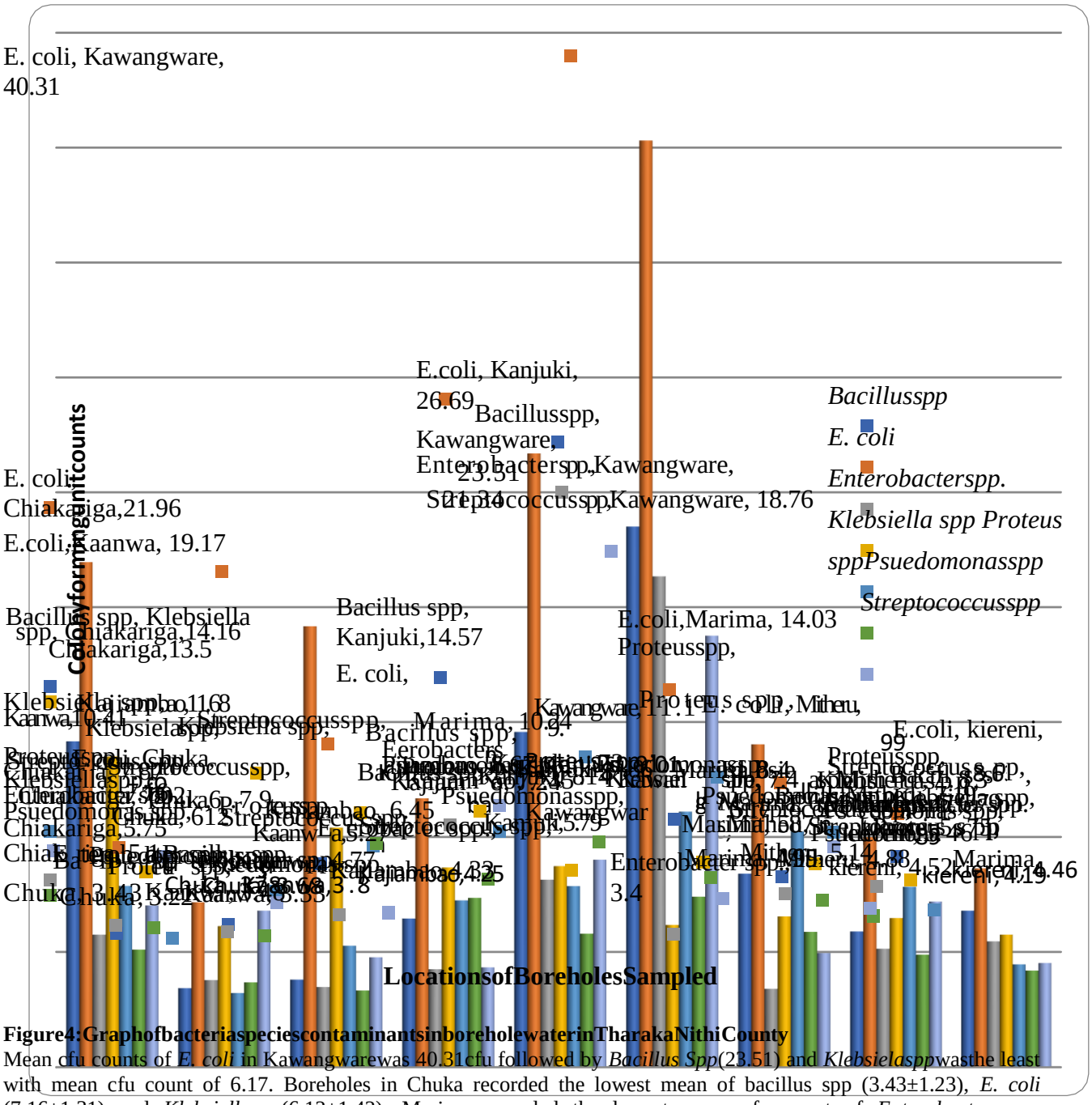

Figure4:Graphofbacteriaspeciescontaminantsinboreholewaterin TharakaNithiCounty

Mean cfu counts of *E. coli* in Kawangwarewas 40.31cfu followed by *Bacillus Spp*(23.51) and *Klebsielaspp*wasthe least with mean cfu count of 6.17. Boreholes in Chuka recorded the lowest mean of bacillus spp (3.43±1.23), *E. coli* (7.16±1.31) and *Klebsiellaspp*(6.12±1.42). Marima recorded the lowest mean cfu count of *Enterobacter spp.* (3.40±1.42). However, *Klebsiellaspp*highest mean cfu count (13.50±1.33) was recorded in Chiakariga. Means and standard deviations of respective boreholes in different locations and respective bacteria species contaminants are presented in **table 5**.

Table8:Locations,BoreholesandtheirRespectiveBacteriaspeciesContaminants

Location		*Bacillusspp*	*E.coli*	*Enterobacterspp.*	*Klebsiellaspp*	*Proteusspp*	*Psuedomonasspp*	*Streptococcusspp*
Chiakariga	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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	Borehole1	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
	Borehole2	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
	Borehole3	4.22±1.34	29.45±27	3.0±1	6.952±1.732	3.557±1.343	3.0±1	3.0±1
	Borehole4	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
Kajiampau	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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
Kanjuki	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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
Mitheru	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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	Borehole1	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
	Borehole2	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
	Borehole3	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
	Borehole4	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

Table9:PhenotypicandBiochemicalcharacteristicsofBacteriaIsolatedfromBoreholewaterinTharakaNithiCounty

Isolates	Colonycharateristics	Gramstain	Catalase	Oxidase	Coagulase	Indole	Citrate	Ureas	VP	Species
1	SmallCreamy,rough surface	+verod chain	-ve	-ve	-ve	-ve	+ve	+ve		*Bacillusspp*
2	Medium greenish, entire margin, low convex	-verodsingle	+ve	+ve	-ve	-ve	+ve	-ve	+ve	*Psuedomonasspp*
3	largeCreamy,rough surface	-verodsingle	+ve	-ve	-ve	-ve	+ve	+ve	+ve	Klebsiellaspp
4	Medium cream, convex, smooth margin	-verodsingle	+ve	-ve	-ve	+ve	-ve	-ve	+ve	*E.coli*
5	Cremi,circular,shiny	- cocci	+ve	-ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus spp.*
6		-Rodshaped	+ve	-ve	-ve	+ve	-ve	+ve	-ve	*Proteusspp*
7			+ve	-ve	-ve	-ve	+ve	+ve	+ve	*Enterobacterspp.*

DISCUSSION

Higher total coliformcount that exceeds the WHO international standards (<1 coliform/100 ml) was observed in the entire borehole sampled across TharakaNithi County. 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 thermotolerant (fecal) coliforms due to organic matter or soil or organic entry in water. The higher levels of total coliform observedmay be as a result of elevated temperature experiencedin Tharaka Nithi County. As pointed out byBello *et al.* (2013), proliferation of microorganismsmaybe affected by variation in prevailing temperatures. Positioning and distance of pit latrines dug a few meters from the boreholes may be linked to total coliform counts due to underground leaching from biosolids.

Depth of boreholes may also contribute to water contamination due to insufficient bacteria filtration (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 waterflow into the well as explained in work of Palamuleni and Akoth (2015), Obioma *et al*. (2018) and Ayika*et al*. (2019). Areas of Kathwana where Kajiampao, Chiakariga and Kawangware are found to have sandy and rocky ground that may allow microorganisms to sip into the well and boreholes.

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 pathogen in the water samples. 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.Our finding on higher contamination of borehole water with feacal coliform corroborates to those of (Orotho and Fwej, 2012). However,our findings differ with those of Oria-Usifo*et al.* (2018) which reported no feacal coliform in borehole water. All the wells surveyed reported cases of pathogenic bacteria. *K. pneumoniae* was isolated in all the sample locations (100%), thermotolerant *E. coli* was isolated in the samples from location III in addition to *K. pneumoniae*, while *Proteus vulgaris* was isolated in the samples from locations IV and V in additionto*K.pnemoniae*.*Salmonella*,*Shigella*,or*Vibrio* werenot isolated fromanyofthe well watersamples. Thepresence of thermotolerant *E. coli* and *K. pneumoniae* indicated fecal contamination of ground water with human or animal sewage. This poses a potential hazard of transmission of waterborne diseases by these pathogenic organisms

Our study finding corroborates with other findings from elsewhere which also reported occurrence of pathogenic bacteria in borehole and wells used for domestic water supply (Ortiz, 2007; Palamuleni and Akot., 2015). Different wells were found to be contaminated with *Escherichia coli* animal or human fecal bacteria suggesting possible presence of other disease causing pathogens. Presence of *E. coli* is a health concern for the development of diseases both in children and adult (World Health Organization, 2017). *Klebsiella* sppa thermotolerant coliforms occurred in most well sampled .*Klebsiella*spp inhabits various natural environment with ability to multiply water containing high nutrients (World Health Organization, 2017). Occurrence of *Klebsiella* spp is an indication that the wells may be contaminated with feacal wastes. Nonetheless, *Klebsiella* spp. incidence in water may however, not cause gastrointestinal illness when ingested in water (Ainsworth and Organization, 2004). The *Proteus* spp a members of the enterobacteriaceae was also isolated fromborehole water samples in this study.*Proteus*spp may have originated fromeither natural habitatofhuman waste.Consumptionof water contaminated by*Proteus*spp putinfants,children and immune compromised individual at risk of contracting infection. Furthermore, Bacillus (16.72%) is associated with a variety of infectious diseases such as gastrointestinal tract, urinary tract and respiratory tract infection [29].

CONCLUSION

Vulnerability of boreholes to contaminants is a health concern in Tharaka Nithi County especially boreholes which are used as source of drinking water.

RECOMMENDATIONS

There is need to educate households in Tharaka Nithi who depend on borehole water on health concerns and theneed to properly treat water prior to usesince most people uses borehole water without any treatment, affordable treatment methodssuchasboilingor chlorination. Thereisneed to educatecommunityon positionand safelocation 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.

REFERENCES

Ainsworth, R., & Organization, W. H. (2004). *Safe, Piped Water: Managing Microbial Water Quality in Piped Distribution Systems.* Geneva, London: IWA Publishing, World Health Organization.

Anyamene,N.C.,&Ojiagu,D.(2014). BacteriologicalAnalysisof sachetwatersoldinAkwaMetropolis,Nigeria. *InternationalJournalofAgricultureandBiosciences,3*,120–122.

Arvanitidou, M., Kanellou, K., Constantinides, T., &Katsouyanno-poulos, V. (1999). The occurrence of fungi in hospital and community potable waters. *Lett. Appl. Microbiol., 29*, 81-84.

Ayanbimpe, G. M., EyiojoAbbah, V., &Ior, C. A. (2012). Yeasts and yeast-like fungalcontaminants of water used for domestic purposes in Jos,Nigeria. *Microbiology Research, 3:e24*, 99-102.

Ayika, P. D., Danladi, M. M., Istifanus, M., &Yenkat, G. (2019). Bacteriological Quality of Boreholes And Wellsin Bokkos, Plateau State Nigeria. *Journal Of Pharmacy And Biological Sciences, 14*(4 Ser. I), 66-71.

Babič, M. N., Gunde-Cimerman, N., Vargha, M., Tischner, Z., Magyar, D., Veríssimo, C., . . . Brandão8, J. (2017). Fungal Contaminants in Drinking Water Regulation? A Tale of Ecology, Exposure, Purification and Clinical Relevance. *Int J Environ Res Public Health, 14*(6), 636. doi:10.3390/ijerph14060636

Bartram, Jamie, Ballance, & Richard. (1996). *Water quality monitoring : A practical guide to the design and implementation of freshwater quality studies and monitoring programs / edited by Jamie Bartram and Richard Ballance. London : E & FN Spon.* (J. Bartram, & R. Ballance, Eds.) London: E & FN Spon. Retrieved from https://apps.who.int/iris/handle/10665/41851

Bello, O., Osho, A., Bankole, S., & Bello, T. (2013). Bacteriological and physicochemical analyses of borehole and well water sources in Ijebu-Ode, Southwestern Nigeria. *Int. J. Pharm. Biol. Sci., 8*, 18–25.

Brink,B.(2016).*UreaseTestProtocol.*USA:AmericanSocietyofMicrobiology.

Dara, S., & Mishra, D. (2011). *A textbook of Environ-mental Chemistry and Pollution control.* New Delhi : Chand and company ltd.

DEFRA(Department for Environment, F. &. (2011). *A Review of Fungi in Drinking Water and the Implications for Human Health* (1st ed.). Paris, France: BIO Intelligence Service.

DevelopmentInitiatives,(.(2018).*enhancingaccesstosafewaterandimprovedsanitationservicesinKenya.* Nairobi:DevelopmentInitiatives.

Doggett,M.(2000).Characterisationoffungalbiofilmswithinamunicipalwaterdistributionsystem.*Appl.Environ. Microb.,66*,1249–1251.

Gunhild,H.,Ann,K.,Peter,G.,Sybren,d.H.,&Ida,S.(2006).DiversityandsignificanceofMoldspeciesin Norwegian drinking water. *Appl. Environ. Microbiol., 72*(12), 7586-7593.

Hassan, B. A. (2019). Carbohydrate fermentation test & starch hydrolysis test. doi:10.13140/RG.2.2.21943.57762
Hoog,
G. S., Guarro, J., Gené, J., &Figueras, M. J. (2000). *Atlas of clinical fungi* (2 nd ed.). (G. S. Hoog, J. Guarro, J.Gené,&M.J.Figueras,Eds.)

Idibie, O. C., Oviojie, O. E., Isalar, O. F., &Emoghene, A. O. (2018). Comparative Microbial Analysis of Borehole Water and other Sources of Water in Benin Metropolis, Edo State. *Journal of Environmental Science and Public Health, 2*, 232-242.

Isikwue, M. O., & Chikezie, A. (2014). Quality assessment of various sachet water brands marketed in Bauchi metropolis of Nigeria. *International Journal of Advances in Engineering and Technology, 6*, 2489–2495.

Moyo, N. (2013). An analysis of the chemical and microbiological quality of ground water from boreholes and shallow wells in Zimbabwe. Phys. Chem. Earth. 2013, 66, 27–32. *Phys. Chem. Earth, 66*, 27–32.
Obioma, A., Chikanka, A. T., & Loveth, N. W. (2017). Evaluation of Bacteriological Quality of Surface, Well, Borehole and River Water in Khana Local Government Area of Rivers State, Niger Delta. *Ann Clin Lab Res., 5*(3), 183.

Obioma, A., Nnenna, I., & Golden, O. (2018). Bacteriological Risk Assessment of Borehole Sources of Drinking Water in Some Part of Port Harcourt Metropolis of Niger Delta, Nigeria.*Biomedical Journal of Scientific& Technical*
Research . doi:10.26717/BJSTR.2020.24.004093

Obot,E.,&Edi,D.(2012).SpatialVariationofBoreholeWaterQualitywithDepthinUyoMunicipality,Nigeria. InternationalJournalofEnvironmentalScience.*ManagementandEngineeringResearch,1*(1),1-9.

Ogolla,O. F.,&Neema,D.B.(2019).Cultural,MorphologicalandBiochemicalIdentificationofXanthomonasSpp the Causative Agent of Bacteria Leaf Spot in Tomatoes in Wanguru, Mwea, Kirinyaga County, Kenya. *International Journal of Research and Innovation in Applied Science, IV*(IV), 44-48.

Okpako, E. C., Osuagwu, A. N., Duke, A. E., &Ntui, V. (2009). Prevalence and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria. *African Journal of Microbiology Research, 3*(2), 056-061.

Oludairo, O., &Aiyedun, J. (2016). Contamination of commercially packaged sachet water and the public health implications: An overview. *Bangladesh Journal of veterinary medicine, 13*(2), 73–81.

Onuorah, S., Nwoke, J., &Odibo, F. (2019). Diversity of Fungi in the Public Hand-pump Borehole Water in Onueke, Ezza Local Government Area, Ebonyi State, Nigeria. *Advances in Pharmacology and Pharmacy, 7*(2), 23-32.

Opara,A.U.,&Nnodim,J.(2014).PrevalenceofBacteriainbottledandsachetwatersoldinOwerrimetropolis. *InternationalJournalofScienceInnovationsandDiscoveries,4*,117–122.

Oria-Usifo, E. E., Cole, N. O., E, O. G., & And Iyeke, S. D. (2018). Assessment of Borehole Water Quality in OredoMunicipality. *International Journal of Engineering Science Invention, 7*(10 Ver I), 24-28.

Orotho, P. P., &Fwej, L. W. (2012). *The microbiological quality of wells and borehole waters in Dar es Salaam Region.* The Open University of Tanzania.

Ortiz, R. (2007). Assessment of microbial and chemical water quality of individual and small system groundwater supplies in Arizona. *PhD_Thesis: University of Arizona, Department of Soil and Environmental Science; 2007.*

Palamuleni, L., & Akot, M. (2015). Physico-Chemical and Microbial Analysis of Selected Borehole Water in Mahikeng,SouthAfrica.*Int.J.Environ.Res. Public Health,12*, 8619- 8630. doi:10.3390/ijerph120808619

Palamuleni, L., & Akoth, M. (2015). Physico-Chemical and Microbial Analysis of Selected Borehole Water in Mahikeng, South Africa. *Int. J. Environ. Res. Public Health, 12*, 8619-8630. doi:10.3390/ijerph120808619

Public Health England, (. (2018). *UK Standards for Microbiology Investigations: Coagulase test.* England: the Standards Unit, Public Health England.

Public HealthEngland, (. (2019). *UK Standards for Microbiology Investigations: Oxidation/fermentation ofglucose test.* London. Retrieved from https://assets.pu

Rakotovao-Ravahatra, Z., Randriatsarafara, F., Milasoanjara, R., Ranaivosoa, M., Rakotovao, A., &Rasamindrakotroka, A. (2019). Assessment of the Coagulase Test in the Identification of Staphylococcus Aureus Strains. *J BiotechnolBiomed , 2*(3), 105-111. doi:10.26502/jbb.2642-91280014

Sebiawu, G. E., Asiedu, S., & Saana, M. (2014). A Physico-Chemical and Bacteriological Analysis of Borehole Water Samples from the Wa Municipality of the Upper West Region, Ghana. *International Journal of Engineering Research and Technology, 3*(5), 1959-1965.

Seth,O.,Tagbor,T.,&Bernard,O. (2014).Assessmentof chemicalqualityof groundwater over somerock types in Ashanti region, Ghana. *Am. J. Sci. Ind. Res., 5*, 1–6.

Shields,P.,&Cathcart,L.(2016). *OxidaseTestProtocol.*USA:AmericanSocietyofMicrobiology.

Sila, O. N. (2019). Physico-chemical and bacteriological quality of water sources in rural settings, a case study of Kenya, Africa. *Scientific African, 2*, 1-13. Retrieved from https://doi.org/10.1016/j.sciaf.2018.e00018

Ugwuzor, N. U., & Ifeanyi, O. E. (2015). Bacteriological Assessment of Different Borehole Drinking. *International Journal of Current Microbiology and Applied Sciences, 4*(5), 1139-1150.

Warris, A. (2003). Hospital water- is it safe? Invasive asspergillosis and hospital water. *13th European Congress of Clinical Microbiology and Infectious Diseases, 13 May.* Glasgow.

World Health Organization, (. (2017). *Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum.* Geneva: World Health Organization. Retrieved from https://www.who.int/publications/guidelines/en/

WRA.(2018).*WaterResourceSituationReport.*Nairobi:KenyaWaterResourceAuthority(WRA).

****