

Bacteriological Quality of Water from Selected Water Sources in Samburu South – Kenya

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Abstract: Water is the most critical resource in the Samburu District of Northern Kenya. The region has one permanent river, the Uaso Ng'iro. Use pressure by man, domestic and wild animals is high in all water sources, which include dams, laggas, and dry river bed wells. The purpose of this study was to investigate the probable causes of perpetual diarrheal diseases in Samburu. Samples were collected quarterly over 1 year. A total of 207 water samples were collected and their microbial quality determined based on the most probable number (MPN) of coliforms, total plate count, fecal coliform counts and presence or absence of *E. coli*. To isolate water borne bacterial pathogens, samples were inoculated in to appropriate enrichment and selective media and the recovered bacteria characterized using relevant biochemical and serological tests. On the basis of total plate count 85% of the water samples were unfit for human consumption. Mean MPN was highest in dams at 643coliforms mL⁻¹ and lowest in springs and boreholes at 35coliforms mL⁻¹. As such, the water from dams, rivers, springs, laggas and dry river bed wells was unfit for human consumption. However, on the basis of presence of fecal coliforms, all water sources did not meet the standards for potability. The bacterial pathogens isolated were *Shigella flexineri*, *Shigella boydii*, *Aeromonas hydrophilla*, and *Salmonella spp.* (non-typhi). *Klebsiella* and *Pseudomonas spp.* considered to be of low clinical significance, were isolated from springs and permanent rivers. Although boreholes and springs had better quality water, Samburu District water did not meet the WHO and KEBS requirement for potability. Monitoring should be carried out over a longer period of time using more rapid and discriminatory procedures. Appropriate and affordable water disinfection techniques such as boiling and filtration are recommended.

Key words: water, quality, bacteria, Samburu

1. INTRODUCTION.

Waterborne pathogens often cause diarrheal disease, a serious international problem. Poor water quality continues to pose a major threat to human health. Diarrheal disease alone amounts to an estimated 4.1% of the total global burden of diseases and is responsible for the deaths of 1.8 million people every year [23]. 88% of this burden is attributable to unsafe water supply, sanitation and hygiene and is mostly concentrated on children in developing countries. 2 million children under the age of five die every year from diarrhea. Most of these diarrheal cases are caused by use of unsafe drinking water and poor sanitation [5]. Lack of access to safe water and sanitation contributes to diarrheal morbidity and mortality in developing countries (Garret *et al.*, 2008). WHO estimates that in 2002, 38% of Kenyans lacked access to safe drinking water. However, when looking only at rural areas, this number increases to 54% [23]. Poor water supplies, sanitation and hygiene pose a major threat to the health of children under the age of five years. Even a nonfatal diarrheal disease is a critical concern, especially for children. Diarrheal diseases among children under the age of five years, account for over 4.7% of all outpatient cases country-wide with the annual incidence of diarrhea being 3.5 to 4.6 episodes per child per year, making it one of the top child killers [17]. The fourth Millennium Development Goal is to reduce child mortality. The target is to reduce by two thirds the mortality rate among children under the age of five by the year 2015. Diarrhea has lifelong effects on children, leading directly to a decrease in physical and cognitive development[4]. In the 2005 – 2015 “Water for Life Decade”, there has been a general shift in water issues quantity alone, with the World Water Day on 22nd March 2010, campaign being to raise the profile of water quality (WHO and UNICEF, 2010).

Water is the most critical resource in the Samburu region of North-Central Kenya. Samburu District is classified as arid to semi-arid and receives a mean annual rainfall of between 250 mm and 500 mm. The relative humidity is typically low. The mean annual potential evapotranspiration exceeds 2000 mm [11]. The Uaso Ng'iro is the only permanent

river in the district. Additionally, numerous ephemeral laggas, which are water channels formed during the rainy season, and natural ponds, have water during the rain seasons (March to May and October to December). Man made dams for harvesting rainwater augment the water resources available for domestic, livestock and wildlife use. The study area was Samburu South, which is part of Samburu District situated in Rift Valley province of Kenya (Figure1). Samburu lies between latitudes 0° 40' and 2° 31'S and longitudes 36° 2' and 38° 10'N [2]. The district covers an area of 20.804 Km² from the Southern shores of Lake Turkana, across Samburu, to Isiolo. Eighty four percent of the area is described as low potential and is semi arid.

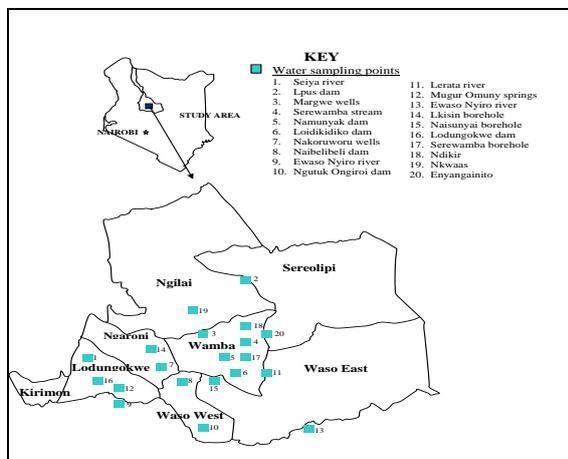


Figure 1. Map of the Samburu District showing the study area (Wamba Division)

In the Samburu region, animals will tend to be dispersed [9]. With the onset of the dry season, animals will tend to migrate to areas with adequate forage and water. Over abstraction of river Uaso Ng'iro water and sinking of boreholes for the purpose of irrigation water supply further reduces the overall amounts of surface water available for man, livestock and wildlife. As man will tend to dominate watering points, competition for available water will inevitably lead to increased human-wildlife conflict, especially at the end of the rain season and during the dry season. As the standing bodies of water start to dry, there will be a corresponding change in water quality [15] and the concentration of dissolved solutes will increase. Most of the Samburu community consumes water directly without any special treatment or filtration procedures being employed on it. Similarly several cases of diarrheal diseases have been reported in Samburu district. Owing to the scarcity of water and sharing of watering points between man, domestic stock and wild animals definitely the

water can be a good vehicle for infective bacteria and other disease causing biological agents. Information on the bacteriological quality of water sources in Samburu District is scanty if any. Given that the majority of people in Samburu consume water directly without any treatment and that causes of diarrheal diseases are common in Samburu there was need to document the water quality of various sources. In Samburu, both ephemeral and permanent water sources are shared between livestock, wildlife and man, and can easily serve as vehicles for transmission of diseases. This study was therefore designed to assess the suitability of available water sources for domestic use by the Samburu community.

2. METHODS.

Purposive sampling targeting water sources for man, livestock and wildlife was carried out. At each water source, whenever possible, water samples were collected directly in pre-sterilized polypropylene bottles of 500 mL. At the water reservoirs, the bottles were opened aseptically, then held at their bases and submerged to a depth of about 20 cm with the mouth facing upwards. Samples were taken by filling the bottles to the top to exclude air. In case of a current, the bottles were tilted towards the current and filled. The samples were examined within 1 – 3 h. of collection. Where sources were too shallow, a water scooper was used to fill the bottles, which were then labeled and carried for further laboratory analysis. Where delays of 3 – 6 h. were anticipated, the bottles were kept on ice, preferably in an icebox until the time of analysis.

Total plate count

An amount consisting of 1 mL of each water sample was transferred aseptically onto sterile petri dishes and approximately 20 mL of molten plate count agar (45°C) added and mixed. The plates were allowed to set then incubated at 37°C for 48 h. (Method 9215B, [1]. Water samples that looked more polluted (>300 counts/mL) were serially diluted using peptone water or physiological saline before plating. Colony counts were made from plates with less than 300 but more than 30 colonies and results expressed as actual colony count multiplied by the dilution factor. Colony counts were expressed as colony forming units (cfu)/mL⁻¹ of the sample.

Coliform tests

Coliforms were enumerated using the most probable number (MPN) of bacterial in water [16]. A series of lauryl tryptose broth (LTB) fermentation tubes were inoculated with 10, 1 and 0.1 mL of the sample (Method 9200A,)[1].

Formation of gas at 35°C within 48 h. constituted a positive presumptive test. To confirm the test, inocula from positive tubes were transferred to tubes of 2% brilliant green bile lactose broth (Oxoid), dispensed in fermentation tubes fitted with inverted Durham tubes and incubated at 37°C for 24 h. Production of gas was taken as a positive test for the presence of coliforms.

Faecal coliforms

A small proportion of the culture from each positive presumptive tube was recultured in brilliant green bile lactose broth 2% (Oxoid) in fermentation tubes and incubated in a water bath at $44.5 \pm 0.2^\circ\text{C}$ for 24 h. (Method 9222D) [1].

Test for *Escherichia coli* A loopful of the positive broth was streaked onto Eosin Methylene Blue agar (EMBA) and incubated for 24 h. at 37°C. The test was completed by making a thin smear for Gram staining from the green metallic sheen colonies and confirmed by biochemical tests (Method 9223A)[1]. Differentiation of coliform into subgroups was carried out on the basis of the result of four tests; Indole, Methyl red, Voges Proskauer and Citrate utilization, often referred to collectively as "IMVIC" tests.

Faecal Streptococci

100 mL of a water sample was filtered through sartoious memberane filters with a pore size diameter of 0.45 μm , and the filter transferred aseptically onto the Slanetz and Bartley agar (Oxoid), plate using sterile forceps. The plates were then incubated at 37°C for 24 h. After incubation, the plates were observed for typical maroon colonies which were counted and recorded as faecal streptococci per 100 mL of water sample.

Isolation of bacterial pathogens

Commenced upon arrival at the Mombasa Polytechnic University College microbiology laboratory. Water samples were plated on different selective media which included XLD agar (HiMedia), for non-lactose fermenting bacteria, Selenite F. broth (HiMedia), for *Salmonella spp.*, MaConkeys agar (Difco), for non-lactose fermenting enterobacteria, SS agar (Difco), for *Salmonella* and *Shigella*, TCBS broth (Oxoid), for *Vibrio* and *Aeromonas* and Campy-blood free agar (Oxoid), for *Campylobacter jejuni*. The plates were then incubated over night at 37°C, except for the campy-blood free agar, which was incubated for 48 h. in a microaerophilic environment at 37°C.

Identification of *Salmonella* and *Shigella*

Water samples were cultivated on selective and differential substrates in order to isolate *Salmonella* and *Shigella* species. Isolated colonies were further subjected to biochemical and serological tests for confirmation of genus. The

water samples were plated on MacConkeys agar (Difco), Xylose lysine deoxycholate agar (HiMedia), *Salmonella Shigella* agar (SS), and Selenite F agar using a cotton swab and incubated overnight at 37°C. MAC, XLD and SS plates were examined for typical non-lactose fermenting colonies. Such colonies were identified by morphology. Suspected colonies were inoculated into TSI agar (Difco), MIO agar (Oxoid), Simmon's Citrate agar (Oxoid), and Urea agar slopes and incubated at 37°C for 12 - 18 h. The selenite F broth was subcultured on to XLD and SS agar plates and incubated at 37°C for 12 - 18 h. Test results were examined by inoculating one set of API 20E (BioMerieux), system according to manufactures instructions, and slide agglutination.

Detection of vibrios and aeromonads

Thiosulfate Citrate Bile salts Sucrose agar (Oxoid), was inoculated with 0.1 mL of 100 mL water samples centrifugates. TCBS agar was checked for growth of typical yellow or blue-green colonies. *Vibrio* and *Aeromonas spp* were identified by the following tests: oxidase, motility, sensitivity to the vibriostatic agent O129 and finally confirmed by API 20NE (BioMerieux) system and serology.

3. RESULTS.

Total coliform numbers expressed as the most probable number (MPN) per 1 mL of water ranged from 4 in boreholes and springs to $>2400 \text{ mL}^{-1}$ in dry river bed wells, permanent rivers, dams and laggas (Figure 2). Mean MPN per mL of water was highest in dams at 643 ± 444 and lowest in springs at 35 ± 186 (Table 1). Based on a one way ANOVA test it was found that there was a significant difference in MPN of water from the different categories of water sources ($P=0.005$, $df=39$). Mean separation using the Tukey- Kramer test revealed that the significant difference in MPN occurred between dry river bed wells and dams (Table 1).

Total plate counts (TPC) in water from the various sources ranged from 2 cfu mL^{-1} in permanent rivers to 240 cfu mL^{-1} in laggas (Figure10). Mean TPC ranged between 13 ± 7 in boreholes to 241 ± 27 in laggas. A one way ANOVA test revealed a significant difference in TPC of water from the different categories of water sources ($P<0.001$, $df = 39$). Mean separation using the Tukey Kramer test revealed that the significant difference occurred between laggas and all the other categories of water sources (Table 1).

Table 1. Mean counts of selected water quality indicators in different categories of water sources investigated in Samburu South.

Source	MPN	TPC	F coli 44
Dry River Beds	485 ^b ±438	71 ^b ±55	16±14
Permanent Rivers	504 ^b ±417	51 ^b ±52	9±8
Dams	643 ^a ±444	89 ^a ±67	21±18
Laggas	186 ^b ±370	241 ^a ±27	21±13
Boreholes	35 ^b ±64	13 ^b ±7	1±2
Springs	35 ^b ±186	24 ^b ±12	4±3

a-b: The same alphabet on different sample shows no significant difference ($p > 0.05$).

The most probable number of bacteria per 1 mL of water ranged from 4 in boreholes and springs to >2400/mL in dry river bed wells, permanent rivers, dams and laggas (Figure 2). Based on a one way ANOVA test, it was found that there was a significant difference in the MPN of water from the different categories of sources ($P = 0.005$, $df = 39$). Mean separation using the Tukey- Kramer test revealed that the significant difference in MPN occurred between dry river bed wells and dams.

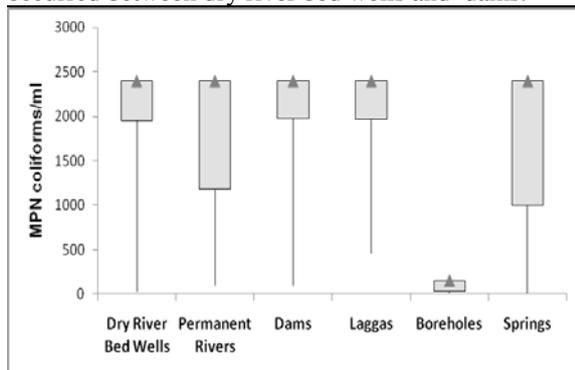


Figure 2. Most probable numbers (MPN) of coliforms in water from different categories of water.

Source	RvrBed Wells	Perm rvrs	Dams	Laggas	Springs	B/hole
Present	20	7	17	8	3	0
Absent	8	13	3	4	7	6
Total	28	20	20	12	10	6

Total plate counts ranged from 2 cfu mL⁻¹ in permanent rivers to 240 cfu/mL⁻¹ in laggas (Figure 3). A one way ANOVA test revealed a significant difference in TPC of water from the different categories of sources ($P < 0.001$, $df = 39$). Mean separation using the Tukey Kramer test revealed that the significant difference occurred between laggas and all the other categories of water sources (Table 1).

Table 2. Frequency of occurrence of *E. coli* in water from different categories of water

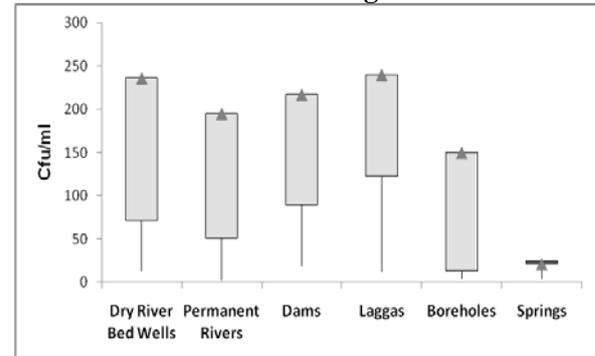


Figure 3. Total plate counts (TPC) in different categories of water sources in Samburu South

The highest number of faecal coliforms per 1 mL of water of 75 cfu mL⁻¹ was recorded in a dam sample, while some water samples from springs, boreholes, dry rivers beds and permanent rivers registered no faecal coliforms. Mean faecal coliform counts were lowest in boreholes(1) and highest in dams (21), which are temporary water source(Figure 4). Using a one way ANOVA test revealed that the mean faecal coliform counts in water from the different sources was insignificant ($p = 0.726$, $df = 39$).

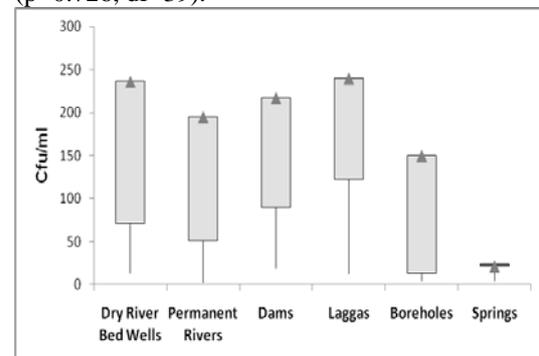


Figure 4. Number of faecal coliforms in water from different categories of water sources in Samburu South.

The frequency of occurrence of *E. coli* in water from the different sources varied widely (Table 2). The highest occurrence was among the dry river bed wells with *E. coli* occurring in 20 out of the 28 wells, followed by dams with 17 out of the 20 dams. The least occurrence of *E. coli* was in springs, where they were present in 3 out of 8 spring water sources. *E. coli* was not detected in boreholes.

Pathogenic Bacterial Isolates in water from various sources

S. flexineri was isolated in 19 dry river bed wells, 12 dams and 2 laggas. *A. hydrophilla* was

recovered in 2 dry river bed wells, 1 permanent river, 2 dams and 1 laggas (Figure 5).

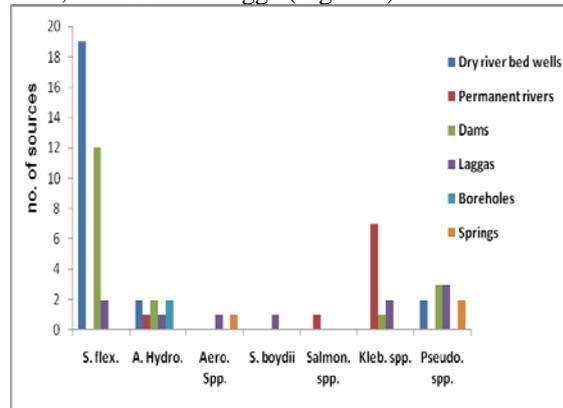


Figure 5. Number of water sources in each category from which specific pathogens were recovered.

On the other hand, water from 16 sources, (boreholes and springs) registered the presence of *Pseudomonas spp.*, *Aeromonas spp.* and had the least frequency of occurrence of pathogenic bacterial isolates.

4. DISCUSSION

Indicator organisms

Monitoring the levels of indicator bacteria in water provides a dependable safety factor because of their large numbers in contaminated waters; a feature that has been reinforced over many years of experience (WHO, 2003). On the basis of the most probable numbers of coliforms per mL of water, only borehole water is fit for human consumption. The other sources had MPNs higher than 100 coliforms per mL of water with some having > 2400 coliforms per mL and therefore do not meet WHO and KEBS standards for drinking water.

Based on the total plate count, 85% of the water sources were found to be unfit for human consumption. Overall, only borehole and spring waters were fit for human consumption while the rest do not meet the WHO and KEBS standards for potability. Boreholes by their very nature are physically protected from contamination due to lack of accessibility by man and animals. Spring water also arises from underground and is therefore less likely to be contaminated compared to dams, rivers and laggas which are open to man and animals. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils [10].

According to [23], fecal coliforms should not be detectable in potable water (should record zero cfu per 100 mL of water). Hence the presence of fecal

coliforms in all categories of water sources except boreholes, confirms that most water sources are not fit for human consumption. This therefore means that the direct use of natural water sources in Samburu district poses a health risk to the consumers. The study further revealed that there was a high chance of recovering *S. flexineri* and other pathogens in the presence of high levels of the fecal coliforms and where *E. coli* are present. Out of 45 samples of brown water, 31 showed presence of *E. coli* and 25 of these eventually yielded pathogenic bacteria, whereas of the 22 sources with clear water, only 3 recorded *E. coli*. Similarly pathogens were isolated from 8 sources although only 3 sources yielded pathogens of clinical significance. According to [21], *E. coli* is a good indicator of fecal contamination, provides conclusive evidence of recent fecal pollution, and should not be present in drinking water.

Pathogenic bacteria

According to the guidelines for drinking water quality, there is no tolerable lower limit for pathogens in water intended for consumption, preparing food, drink or for personal hygiene; it should contain no agents pathogenic to humans [22] and [14]. In the present study, *S. flexineri* was recorded in dams, laggas and dry river bed wells. Pathogenic isolates of very minimal clinical significance (*Klebsiella* and *Pseudomonas spp.*) were recorded in 70% of permanent rivers, springs and boreholes. *Pseudomonas spp.* is in the normal micro flora in human and animals [20]. According to [12], *Pseudomonas* does not harm a healthy individual but may cause problems in individuals with weak immune systems. However, it is more reliable and safe if the drinking water does not show the presence of *Pseudomonas spp.* According to [19], the presence of *Pseudomonas spp.* in the water is due to contamination by humans themselves.

Presence of *Shigella* in water sources of Samburu indicated continued fecal contamination. This is because *Shigella* survives up to 4 days in river water. It is improbable that *Shigella* can be recovered from an environmental source, unless there is a continuous source of contamination such as wastewater seepage. *Shigella* can survive in a viable but non culturable state after 21 days [6]. *Salmonella non typhi* were recorded only in permanent rivers and their isolation may have been due to incidental contamination, since the organism had not been recovered in samples collected much earlier during this study.

A. hydrophila and other *Aeromonas spp.* were recorded in all water sources except boreholes. Animals may also be the source of contamination by *A. hydrophilla* [13]. Wildlife is not only abundant in Samburu, but share the watering points with man. Enteric pathogens are likely to be

introduced into the water sources from fecal matter [8]. The Samburu live in temporary shelters (Manyattas), and do not set up any special sanitary facilities such as toilets and bathrooms. It is possible therefore for fecal matter to be washed into the water bodies as surface run off during the rainy seasons. *Klebsiella spp.* and *Pseudomonas spp.* are generally abundant in water, and may not be unique to Samburu south[3]. The highest levels of contamination occurred in dams, followed by dry river bed wells, laggas and finally permanent rivers. The extent of deterioration in water quality is in general related to the retention time of the reservoir and its storage capacity in relation to the amount of water flowing into it. Water stored for many months or even years in a dam undergoes deterioration and may be lethal to most life.

5. CONCLUSIONS

Water from all sources excluding boreholes had total plate and coliform counts that exceed the permissible limits for potable water. Water from these sources evidently harbors *E. coli* and fecal coliforms and is therefore unfit for human consumption.

The isolated bacterial pathogens were *Shigella flexneri*, *Shigella boydii*, *Aeromonas hydrophilla*, and *Salmonella spp* (non-typhi) with a higher occurrence in temporary water sources.

The Samburu communities should implement relevant techniques for sterilizing the water before it is consumed. According to [7] and [18], when thermotolerant (fecal) coliform counts in water are in categories of 20 and 2000 thermotolerant coliform concentration per 100 mL of water, the water could still be used for domestic consumption provided that simple physical treatment and disinfection such as filtration and boiling is carried out.

Since only boreholes and springs meet potability standards, more boreholes should be drilled and accessibility to springs should be improved by clearing surrounding bushes to make paths to the springs. Monitoring should be spread over longer periods (3 years) to capture seasonal variability with more replication of samples to increase the precision margin.

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