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Morphological Characterization of Enteric Pathogens in Thiba River of Kirinyaga County, Kenya

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Abstract: **Water is essential for all known life forms;water pollution and the destruction of ecosystems continue to increase. River waters pollution with dangerous microbes, including bacteria, viruses, parasites, as well as fungi, has been on steady increase in the recent past. The major source of microbes in water is faeces from human and other mammals. The study aimed at determining water borne enteric pathogens in the water of Thiba River and its tributaries and health implications to the users downstream. The samples were collected from Thiba River, which is located in Kirinyaga County. A cross sectional sampling design was used in this study. Isolation of bacteria was done on various types of media. Characterization and identification of the isolates was performed by morphological and biochemical methods. The enumeration of enteric bacteria revealed that the number of faecal coliform (*E. coli*) in water was higher than standard set by World Health Organization (WHO). The number of faecal coliform (*E. coli*) was varying in different seasons and at different stages of the river. The health impact of that unsafe drinking water could be the prevalence of waterborne diarrhoeal diseases in the population. A moderate correlation was observed between the Densities of faecal coliform and diarrhoeal cases in the area under study. Such water with a high number of total and faecal coliform could be the potential sources of waterborne bacterial pathogens. As it is evident from the study, 8 bacteria genera were isolated and identified as follows; *Escherichia, Salmonella, Klebsiella, Proteus, Enterobacter, Erwinia, Serratia, Citrobacter* and *Vibrio*. The frequency of isolation of the organisms identified varied as follows; *E. coli* (38%), *Salmonella* (19%), *Klebsiella* (9%), *Proteus* (9%), *Enterobacter* (7%), *Erwinia* (6%), *Serratia* (4%), *Shigella* (4%), *Citrobacter* (3%) and *Vibrio* (1%). The study has demonstrated that Thiba river harbour diverse potentially pathogenic bacteria species. Seventy isolates were obtained, characterized and identified. Thiba River is contaminated with human and animal waste, the contamination is more in the midstream and downstream where human activities are most active. There is contamination of Thiba River during the rainy season and this happens more at the mid and downstream. The results demonstrate the presence of contaminants in the environment and exposure to potential disease causing agents. Emphasis thus needs to be put into the application of proper hygienic practices to reduce the risk of infection especially from food handlers. Efforts by the Kirinyaga county government to avail sanitation facilities in the slum and villages that use the water from Thiba River will prevent contamination of the river by both human and animal waste.**

Keywords: **Water pollution, Coliforms, Enteric pathogens, Thiba river.**

1. INTRODUCTION

Water is essential for all known life forms;, water pollution and the destruction of ecosystems continue to increase. Contamination of water is now a major problem in the global context as a consequence of industrialisation globalization, population growth, urbanisation and warfare combined with increased wealth and more extravagant lifestyles (UN-Water, 2006). River waters pollution with dangerous microbes, including bacteria, viruses, parasites, as well as fungi, has been on steady increase in the recent past (Musyoki et al., 2013). The major source of microbes in water is faeces from human and other mammals. Entry of pathogens into rivers can occur either from a point source, non- point sources or both. Non-point source microbial pollution of rivers occurs from rainwater surface run-offs, storm sewer spillages or overflow.

While point-source pollution comes from discharge of untreated or partially treated effluents from wastewater treatment plants (Donovan et al., 2008). The impact of river pollution on human health depends mainly on the water uses, as well as the concentration of pathogens in the water (Niyogi, 2005).

Water is important ingredient in chemical metabolism that plays a crucial role in the digestion, absorption of food, transportation of nutrients in the body and elimination of waste products via urine (Jafari et al., 2006). Water borne enteric pathogens presents a greater health risk to people using river water for drinking, irrigation of crops eaten raw, bathing, fishing and recreational activities (Hellweger and Masopust, 2008). Kenya standard for drinking water quality states that no Escherichia coli, Shigellae, Pseudomonas aeruginosa or coliforms should be detectable in 250 mL of drinking water (WASREB, 2006). In order to reduce waterborne disease outbreaks World Health Organization (WHO) developed microbiological quality guidelines based on intended water uses. The guidelines stipulate that faecal coliforms (FC) should not exceed 10^3 per 100 mL of water to be used in irrigation of crops that are eaten uncooked, sports fields, and public parks in unrestricted regions (WHO, 1989). Environmental Protection Agency (EPA) standard is stricter, and requires zero FC / 100 ml of water to be used in irrigation of any food crops not commercially processed including crops eaten raw (EPA, 1992).

Enteric bacteria are bacteria in the family Enterobacteriaceae. These bacteria reside normally in the guts of many animals, including humans, and some are pathogenic, causing disease in certain animal species. Many cases of food poisoning are caused by infection with enteric bacteria, as are some more serious conditions, such as the plague. They are Gram negative, facultative anaerobic or aerobic, non-spore forming bacteria that are either motile or non-motile. Some members of Enterobacteriaceae such as Escherichia coli, Enterobacter, Serratia are natural inhabitants of the gastrointestinal tract of human beings and can be used as indicators of faecal contamination of the environment. The pathogenic members of Enterobacteriaceae that infect the gastrointestinal tract of humans include Salmonella spp, Shigella spp, Proteus spp, Campylobacter and E. coli. They get access to the human GIT when ingested through contaminated water, food and oral contact with infected surfaces (WHO, 2003).

River water is open to many polluting agents especially those waters which gain direct entry of discharges from urban centres. Some of these polluting agents at certain levels may be lethal to both flora and fauna that depend on such waters. Thiba River, like many other rivers in sub-Saharan Africa, is facing serious water pollution problems due to increased discharge of industrial, commercial and domestic effluents into the river system. Users downstream however, pumps and uses raw water from this river for drinking, cooking, bathing, and other uses without much concern of the health implication it would pose to them. The communities downstream use the raw water to irrigate their vegetables some of which are eaten raw. Therefore, the aim of this study was to determine water borne enteric pathogens in the water of Thiba River and health implication to the users downstream that consume this water.

2. MATERIALS AND METHODS

2.1 Collection of water samples:

Water samples were collected from Thiba River which is in Kirinyaga county 50 kilometres southwest of the Nairobi. It is located at 0^o 34' 1.14' S and longitude 37^o 19' 21.30' E. Kirinyaga County is a county in the former Central Province of Kenya. A cross sectional design was used in this study. Various water samples were collected across the river at points that were randomly selected. The samples were transported to the laboratory in ice packed cooler box and analysed within two hours.

2.2 Isolation of enteric pathogens:

Pathogenic enteric bacteria, which infect the GIT of man and other warm- blooded mammals, were isolated as follows. 10^0, 10^{-1}, 10^{-2}, 10^{-3} 10^{-4} and 10^{-5} dilutions was made for each of the liquid samples. Undiluted samples were also plated alongside the diluted samples. 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 of the sample as shown in the formula below.

$$\text{Viable cell count(CFU/g soil)} = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \text{ X Dilution factor}$$

2.3 Culturing technique:

1ml of diluted sample was introduced in 9ml of peptone buffered water and to 9 ml of selenite F broth for enrichment. After enrichment, sub-culturing from alkaline peptone water to Salmonella Shigella agar, MacConkey agar and standard one agar for total aerobic count was done and the plates incubated at 37°C for 24 hours aerobically. Plates with colonies having different morphologies were sub cultured on fresh agar plates until pure isolates were obtained. Colony

morphology were used to characterise the microorganisms. Vibrio colonies, which are sucrose fermenting, will appear yellow on TCBS. Salmonella colonies on SS agar appear to have a black centre which is absent in the Shigella colonies. E coli colonies are lactose fermenting and appear as pink colonies on MacConkey agar which when sub cultured on Muller Hinton have a creamy appearance. (Bergey's et al., 1994).

2.4 Characterization of the isolates:

Preliminary characterization was performed using morphological and cultural characteristics as described by (Holt *et al.*, 1994). Morphological identification of the isolate was done under the dissecting and compound microscope to observe cell size, shape and arrangement characteristics after classical staining of bacteria (Bartholomew, 1962). 3% (w/v) KOH test (Gregersen, 1978) was used to determine gram characteristics of isolates. Biochemical tests that were also conducted included; citrate utilization, gelatine liquefaction, methyl red-Voges Proskauer, urease test, nitrate reduction test, motility at 25^{o} C, starch hydrolysis, H_2S production, catalase test, oxidase test and indole production test.

3. RESULTS

3.1 Identification of the isolates:

Morphological characterization was based on classical macroscopic techniques of colour, form, shape, and elevation of pure colonies. Most colonies were able to grow within 2-3 days of incubation at 25 $^{\circ}$C. The colony morphology of the isolates ranged from, flat and filamentous or branching (Table 1). They were smooth or rough and the colour ranged from white to cream and brown (Table 1). The ability of the isolates to excrete extracellular enzymes was tested through hydrolysis of starch, and gelatine. The ability of the isolates to excrete intracellular enzymes was determined through tests on catalase reaction; urease, Voges-Proskauer, hydrogen sulphide production, nitrate reduction, methyl red, citrate utilization, oxidase and motility. The isolates deferred greatly on their ability to excrete various enzymes (Table 1). The colony morphology of the isolates obtained from Thiba River ranged from circular, entire, convex and flat (Table 1). They were smooth or rough and the colour ranged from red or pink, black, white and brown (Table 1) Black and brown colonies on Salmonella-Shigella agar were suspects for Salmonella and Shigella. Twenty-seven isolates that showed smooth, convex, red or pink colonies on MacConkey were lactose fermenters hence were presumptively identified as E. coli. They were stocked for further biochemical analysis. Pink or red colonies on MacConkey that were mucoid were suspected to be Klebsiella. Colonies grew on one hundred and eighty plated samples and were subjected to Gram staining. Presumptive identification of the microorganisms was based on Gram staining and colony morphology on SS and MacConkey media (Table 1). Table 1 shows the biochemical reactions done on the isolates and the identities of the isolates. It indicates that 27 isolates were identified as *E. coli*, thirteen were *Salmonella*, six *Klebsiella*, six were *Proteus*, three constituted *Shigella* while *Erwinia*, *Enterobacter*, *Serratia*, *Citrobacter* and *Vibrio* constituted six, five, three, two and one respectively. Identity of the isolates was confirmed using Bergey's Manual of Determinative Bacteriology.

3.2 Bacterial viable cell count:

The viable cell counts were performed on the bacteria that were able to form visible colonies within 24 hours of inoculation. Water samples were diluted in quarter strength ringer solution, and aliquots from dilutions were plated onto the media in replicates. The amount of variation in colony numbers between replicate plates in counting set decreased with increased dilution factor. The colony forming units were obtained by computing the average among the set of dilution factor (10^{-4}) from different water sources. Bacterial viable cell count. The viable cell counts were performed on the bacteria that were able to form visible colonies within 24 hours of inoculation on standard one and MacConkey agar plates. Water samples were diluted in quarter strength ringer solution, and aliquots from dilutions were plated onto the media in replicates. The amount of variation in colony numbers between replicate plates in counting set decreased with increased dilution factor. The colony forming units were obtained by computing the average among the set of dilution factor (10^{-4}) from different water samples. The rainy season showed highest count as compared to the dry season. The point at which water samples were collected from the river showed that there was less microbial count in the upstream of the river followed by mid-stream while the downstream recorded the highest counts in both dry and rainy seasons. The results also showed that there was statistical support for the differences between the mean counts obtained from upstream, mid-stream and downstream in relation to dry and rainy seasons by one-way analysis of variance (ANOVA) (Table 2 and 3). The faecal coliform count per 1 ml of water samples collected from Thiba river water were higher in the rainy months than in dry months throughout study period. The concentration of enteric bacteria was significantly higher in in rainy season and at the downstream of the river than in the dry season. Data of patients that attended Kerugoya County Hospital between January and April of patients under 5years and above 5 years, showed slight increase in the prevalence of diarrheal disease and gastroenteritis due to consumption of contaminated water reported through doctors. According to the

information, the diarrheal case numbers increased during the rainy season as shown in table 6. The cases were also high in patients under the age of 5 years (Table 4).

4. DISCUSSION

This study aimed at isolating and characterizing dominant water-borne enteric pathogens from Thiba River. The study contributes to the understanding of bacteria diversity and their health implications to the users downstream. Identification of the bacteria was based on morphological and biochemical characteristics. Wide ranges of different media were used to estimate the size of the bacterial community and to isolate specific species of bacteria. Among this media was MacConkey agar that is both selective and differential medium containing lactose, bile salts, neutral red, and crystal violet. Bile salts and crystal violet inhibit growth of Gram-positive bacteria. Neutral red dye is a pH indicator that is colourless above a pH of 6.8 and red at a pH less than 6.8 (Michael and Burton, 2012). Acid accumulating from lactose fermentation turns the dye red. Lactose fermenters turn a shade of red on MacConkey Agar, whereas lactose non-fermenters retain their normal colour or the colour of the medium (Figures 3.1 b and d). Formulations without crystal violet allow growth of *Enterococcus* and some species of *Staphylococcus*, which ferment the lactose and appear pink on the medium (Michael and Burton, 2012).

The second media used, was *Salmonella- Shigella* agar for the isolation of *Salmonella* and *Shigella* from faeces, foodstuffs and other materials. Brilliant green, ox bile and high concentrations of thiosulfate and citrate largely inhibit the accompanying microbial flora. Sulphide production is detected by using thiosulfate and iron ions, the colonies turn black. The presence of coliform bacteria is determined by detecting degradation of lactose to acid with the pH indicator neutral red (Merck, 2005). Standard one agar was used for enumeration of total aerobic and mesophilic counts. These culture media are suitable for the cultivation of fastidious bacteria; after addition of blood, ascites fluid or serum they, can also be used to cultivate streptococci, pneumococci and erysipelas organisms etc. They are used for the enumeration, isolation and enrichment of bacteria and as high-grade bases for preparing special culture media (Merck, 2005).

The taxonomic classification of the isolates performed using morphological characteristics and biochemical tests placed the isolates to the genera *Escherichia, Salmonella, Klebsiella, Proteus, Enterobacter, Erwinia, Serratia, Citrobacter* and *Vibrio.* The frequency of isolation of the organisms identified varied as follows; *E. coli* (38%), *Salmonella* (19%), *Klebsiella* (9%), *Proteus* (9%), *Enterobacter* (7%), *Erwinia* (6%), *Serratia* (4%), *Shigella* (4%), *Citrobacte*r (3%) and *Vibrio* (1%). E. coli had the highest frequency in the studied samples followed by *Salmonella*. Being both pathogenic and part of the normal flora of the GIT of man and most warm -blooded animals, *E. coli* is expected to be found in a large percentage (Balows *et al.*, 1998).

E. coli comprises 1% of the total faecal flora of humans and warm-blooded animals hence sewage is most likely to contain *E. coli* in large numbers (Fricke *et al.*, 2008). Because of poor sanitation of Thiba slums, it is most likely that many of the samples collected could have been contaminated by sewage waste. *E. coli* has in the past been used as an indicator of faecal contamination and presence of other enteric pathogens (Balows *et al.*, 1998). Salmonella and Shigella are pathogenic and can only be found where we have a patient infected by either of them, a carrier or in the case of an outbreak or recent outbreak hence their presence in small percentages in the environment (Shipp and Dickson, 2011). The diseases most frequently associated with water are enteric infections (such as infectious diarrhoea) that are also often associated with food (Mead et al., 1999). In many cases, the disease is relatively mild and self-limiting. However, a proportion of the infected population will suffer more severe outcomes, especially when the health care system is lacking. Several waterborne pathogens, such as *Vibrio cholerae*, *hepatitis E* virus and *Escherichia coli* O157:H7, have high mortality rates (Hunter, 1997).

Thiba River is an important source of water for many residences of Kirinyaga town. The river passes in middle of the town and in the informal settlement such as slums, villages and market places. Therefore, many people depend on this river for house use and for agriculture. The study has shown that the contamination of the river water happens at the midstream and downstream since at these points total enteric and aerobic counts were too high, at this point of the river there are local markets, informal settlements and a lot farming being done along the river. The irony is there are limited sanitary facilities at these places; hence, this could explain high numbers of E. coli and Salmonella isolated. According to WHO, 2003 The number of bacteria in water is a measure of pollution, and the number of bacteria in seawater, groundwater and lake water should be below 100/ml. data from the health centres that service the local people shows that patients come with significant symptoms related to waterborne illness or symptoms of gastrointestinal illness (nausea, diarrhoea, vomiting, abdominal pain). Qualitative and quantitative composition within bacterial pathogens in samples of drinking water of Thiba river were probably due to contamination of sources of drinking water by human or animal excreta

5. CONCLUSION AND RECOMMENDATIONS

Data obtained indicated that water from Thiba River is potential reservoirs of enteric bacteria, posing a potential public health hazard.The targeted genera were E. coli, Salmonella and Shigella. They were isolated in the following proportions: *E. coli* (38%), *Salmonella* (19%), *Klebsiella* (9%), *Proteus* (9%), *Enterobacter* (7%), *Erwinia* (6%), *Serratia* (4%), *Shigella* (4%), *Citrobacter* (3%) and *Vibrio* (1%). The study has demonstrated that Thiba river harbour diverse potentially pathogenic bacteria species. Seventy isolates were obtained, characterized and identified. Thiba River is contaminated with human and animal waste the contamination is more in the midstream and downstream where human activities are most. There is contamination of Thiba River during the rainy season and this happens more at the mid and downstream.

The following are recommendations drawn from this study to aid further research in this area**:**

- The results demonstrate the presence of contaminants in the environment and exposure to potential disease causing agents.
- Emphases thus need to be put into the application of proper hygienic practices to reduce the risk of infection especially from food handlers in this slum area.
- Efforts by the Kirinyaga county government to avail sanitation facilities in the slum and villages that use the water from Thiba River will prevent contamination of the river by both human and animal waste.
- Further analysis of these bacteria is necessary to establish the antibiotic resistance characteristics and their genetic diversity.
- Further studies are needed to isolates and characterize enteric pathogenic bacteria on various food samples that are commonly consumed by the local people.
- More research is required to design studies that would compare the diversity of Bacteria in different seasons of the year such as the rainy and dry seasons.

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APENDIX

Table 1: Biochemical characteristics of enteric bacteria isolated from Thiba River

Strain	Form	Gram reaction	Colour	Margin	Elevation	Opacity	Cat	Oxi	Cit	H_2S	MR	VP	Nit	Ind	Ure	Mot	Microbe Identified
TUI	Contoured	-	Cream	Entire	Raised	Transparent	+	-	+	+	+	+	+	-	+	+	*Proteus mirabilis*
TU2	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TU3	Circular	-	Cream	Entire	Raised	Transparent	+	+	+	+	-	+	+	+	-	+	*Citrobacter diversus*
TU4	Circular	-	Black	Entire	Flat	Opaque	+	-	+	+	+	-	+	-	-	+	*Salmonella* sp.
TU5	Circular	-	Pink	Lobate	Umbonate	Rough	+	+	+	+	+	+	+	-	-	+	*Erwinia* sp.
TU6	Circular	-	Pink	Entire	Convex	Rough	+	-	+	-	-	+	+	-	+	+	*Enterobacter aerogenes*
TM7	Contoured	-	Cream	Lobate	Flat	Transparent	+	-	-	+	+	+	+	+	+	+	*Proteus vulgaris*
TM8	Circular	-	Pink	Undulate	Flat	Opaque	+	-	+	-	-	+	+	-	-	+	*Serratia marcescens*
TM9	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TM10	Smooth	-	White	Entire	Convex	Moist	+	-	+	-	-	+	+	-	+	+	*Enterobacter* sp.
TM11	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TM12	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.

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TD13	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD14	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD15	Mucoid	-	Red	Entire	Raised	Opaque	+	-	+	-	-	+	+	-	+	-	*Klebsiella. pneumonia*
TD16	Mucoid	-	Red	Entire	Slightly raised	Opaque	+	-	+	-	-	+	+	+	+	-	*Klebsiella oxytoca*
TD17	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD18	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD19	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD20	Circular	-	White	Lobate	Umbonate	Rough	+	+	+	+	+	+	+	-	-	+	*Erwinia* sp.
TD21	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD22	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD23	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD24	Circular	-	White	Entire	Convex	Moist	+	-	+	-	-	+	+	-	-	+	*Enterobacter aerogenes*
TD25	Circular	-	Oval	Entire	Convex	Rough	+	+	+	-	-	+	-	-	-	-	*Shigella sp.*
TD26	Contoured	-	Cream	Lobate	Flat	Transparent	+	-	-	+	+	+	+	+	+	+	*Proteus vulgaris*
TD27	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD28	Smooth	-	Pink	Entire	Convex	Moist	+	-	+	-	-	+	+	-	+	+	*Enterobacter* sp.
TD29	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD30	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD31	Circular	-	Red	Entire	Convex	Rough	+	+	+	-	-	+	-	-	-	-	*Shigella sp.*
TD32	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD 33	Contoured	-	Cream	Lobate	Flat	Transparent	+	-	-	+	+	+	+	+	+	+	*Proteus vulgaris*
TD34	Mucoid	-	Red	Entire	Raised	Opaque	+	-	+	-	-	+	+	+	+	-	*Klebsiella sp.*
TD35	Circular	-	Oval	Entire	Convex	Rough	+	+	+	-	-	+	-	-	-	-	*Shigella sp.*
TD36	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TM37	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TM38	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TM39	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TM40	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TU41	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TU42	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD43	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD44	Circular	-	Pink	Entire	Raised	Transparent	+	+	-	-	+	-	+	+	-	+	*Vibrio parahaemolyticus*
TD45	Smooth	-	White	Entire	Convex	Moist	+	-	+	-	-	+	+	-	+	+	*Enterobacter sp.*
TD46	Mucoid	-	Red	Entire	Raised	Opaque	+	-	+	-	-	+	+	-	+	-	*Klebsiella pneumonia*
TD47	Circular	-	White	Entire	Flat	Rough	+	-	+	-	-	+	+	-	+	+	*Serratia sp*
TD48	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD49	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD50	Smooth	-	Red	Entire	Raised	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD51	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD52	Circular	-	Cream	Entire	Raised	Transparent	+	-	+	+	+	+	+	-	+	+	*Proteus mirabilis*
TD53	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TM54	Circular	-	Cream	Entire	Raised	Transparent	+	+	+	+	-	+	+	+	-	+	*Citrobacter diversus*
TM55	Circular	-	Black	Entire	Flat	Opaque	+	-	+	+	+	-	+	-	-	+	*Salmonella* sp.
TM56	Circular	-	White	Lobate	Umbonate	Rough	+	+	+	+	+	+	+	-	-	+	*Erwinia* sp.
TM57	Mucoid	-	Red	Entire	Raised	Opaque	+	-	+	-	-	+	+	-	+	-	*Klebsiella pneumonia*
TM58	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD59	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD60	Contoured	-	Cream	Lobate	Flat	Transparent	+	-	-	+	+	+	+	+	+	+	*Proteus vulgaris*
TD61	Circular	-	White	Lobate	Umbonate	Rough	+	+	+	+	+	+	+	-	-	+	*Erwinia* sp.
TD62	Contoured	-	Cream	Entire	Raised	Transparent	+	-	+	+	+	+	+	-	+	+	*Proteus mirabilis*
TD63	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD64	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD65	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD66	Circular	-	Cream	Entire	Raised	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD67	Mucoid	-	Circular	Swarming	Slightly raised	Opaque	+	-	+	-	-	+	+	+	+	-	*Klebsiella oxytoca*
TD68	Circular	-	Black	Lobate	Slightly raised	Opaque	+	-	+	+	+	-	+	-	-	-	*Salmonella* sp.
TD69	Smooth	-	Red	Entire	Convex	Translucent	+	-	-	-	+	-	+	+	-	+	*Escherichia coli*
TD70	Circular	-	Pink	Entire	Flat	Rough	+	-	+	-	-	+	+	-	+	+	*Serratia sp*

Key: (+) Positive, (-) Negative, MR: Methyl Red, VP: Voges-Proskauer, Oxid; oxidase, Cit; Citrate, Mot; Motility, Ind; Indole; Nit; nitrate; Cit; Ure; Urea and H_2S: Hydrogen Sulphide gas

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Table 2: The mean difference of total aerobic count (CFU/ ml) isolated from standard 1 Agar determined by one- way ANOVA using IBM_SPSS_Statistics_v22 at 95% confidence interval

ANOVA					
		Sum of Squares	Df	Mean Square	F
Dry season	Between Groups	3047295460992908.000	2	1523647730496454.000	94.669
	Within Groups	2221030638297872.000	138	16094424915201.970	
	Total	5268326099290780.000	140		
Rainy season	Between Groups	359358780567375810.000	2	179679390283687904.000	26.965
	Within Groups	919538128936169860.000	138	6663319774899782.000	
	Total	1278896909503545860.000	140		

ANOVA		
		Sig.
Dry season	Between Groups	.000
	Within Groups	
	Total	
Rainy season	Between Groups	.000
	Within Groups	
	Total	

Table 3: The mean difference of total enteric bacteria counts (CFU/ ml) isolated from MacConkey agar determined by one- way ANOVA using IBM_SPSS_Statistics_v22 at 95% confidence interval

ANOVA					
		Sum of Squares	Df	Mean Square	F
Dry season upstream	Between Groups	12880889361702.123	2	6440444680851.062	74.690
	Within Groups	11899634042553.190	138	86229232192.414	
	Total	24780523404255.312	140		
Rainy season Upstream	Between Groups	1515070820821276590.000	2	7575360410638298.000	690.983
	Within Groups	1512916561702127.000	138	10963163490595.129	
	Total	1666363738297872.000	140		

ANOVA		
		Sig.
Dry season upstream	Between Groups	.000
	Within Groups	
	Total	
Rainy season Upstream	Between Groups	.000
	Within Groups	
	Total	

Table 4: Data of patients that attended Kerugoya County Hospital suffering from Diarrhoea and Gastroenteritis of presumed origin from the month of Jan to April

Patients	**Jan**	**Feb**	**Mar**	**Apr**
Under 5 years	145	358	219	188
Over 5 years	80	57	45	148

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PLATES

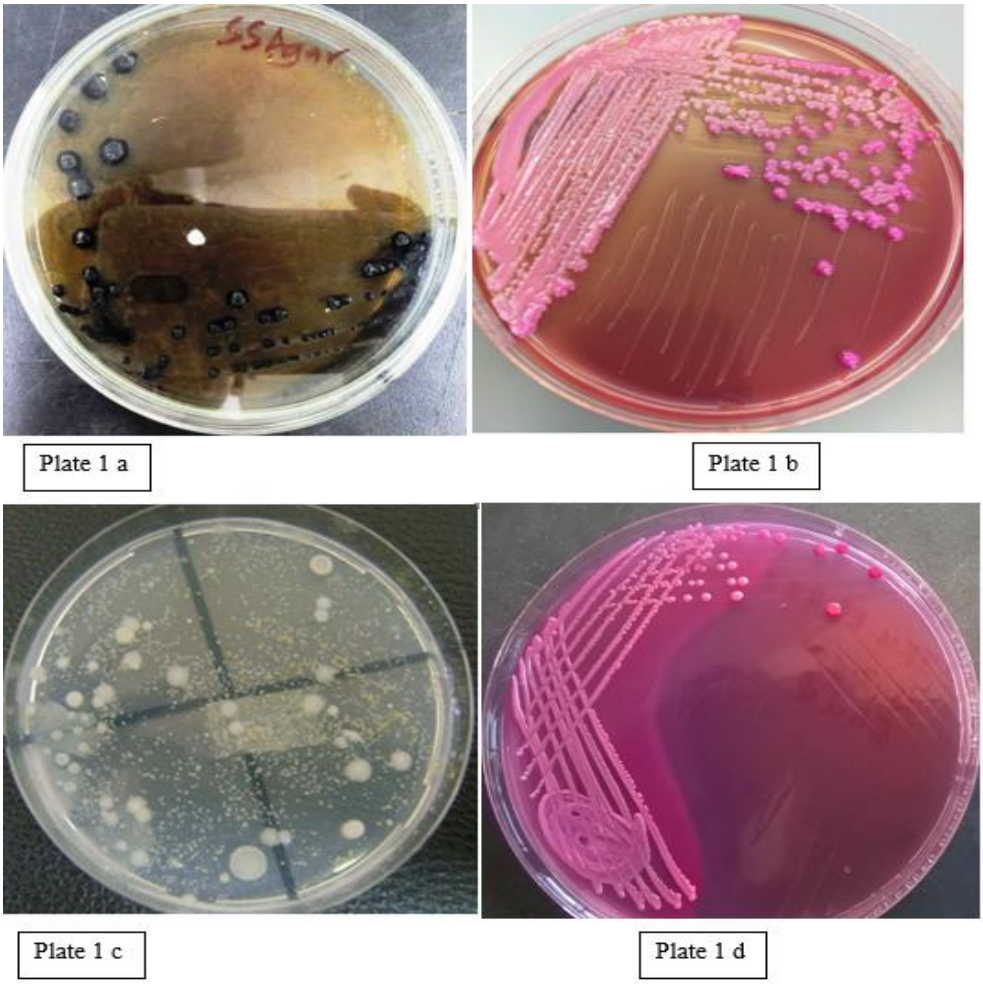

Plate .1: Salmonella Shigella agar plate with pure isolates. (1b) MacConkey agar plate with mixed colonies of lactose and non-lactose fermenters. (1c) Standard 1 agar plate with different aerobic colonies. (1d) MacConkey agar plate with pure isolates of lactose fermenting bacteria

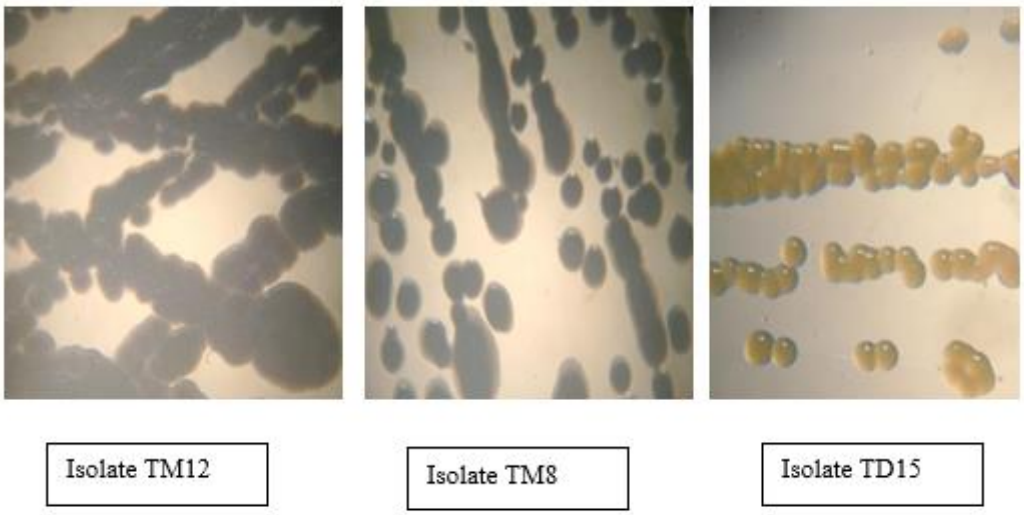

Plate 1: Some of the bacterial colonies, as seen under dissecting microscope

Legend: Circular, raised and lobate (**TM12**), Circular, flat and undulate (**TM8**), Irregular, raised and entire (**TD15**).

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FIGURES

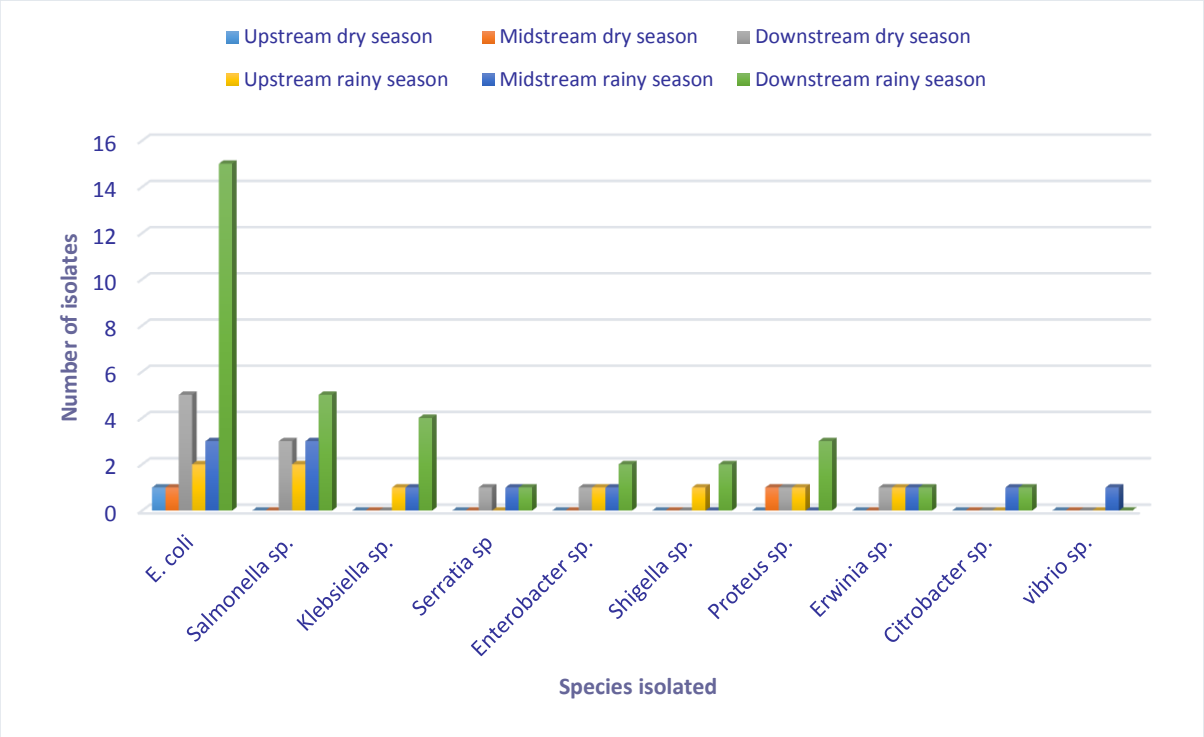

Figure 1: Summary of bacterial species isolated from three stages Thiba River during dry and rainy seasons

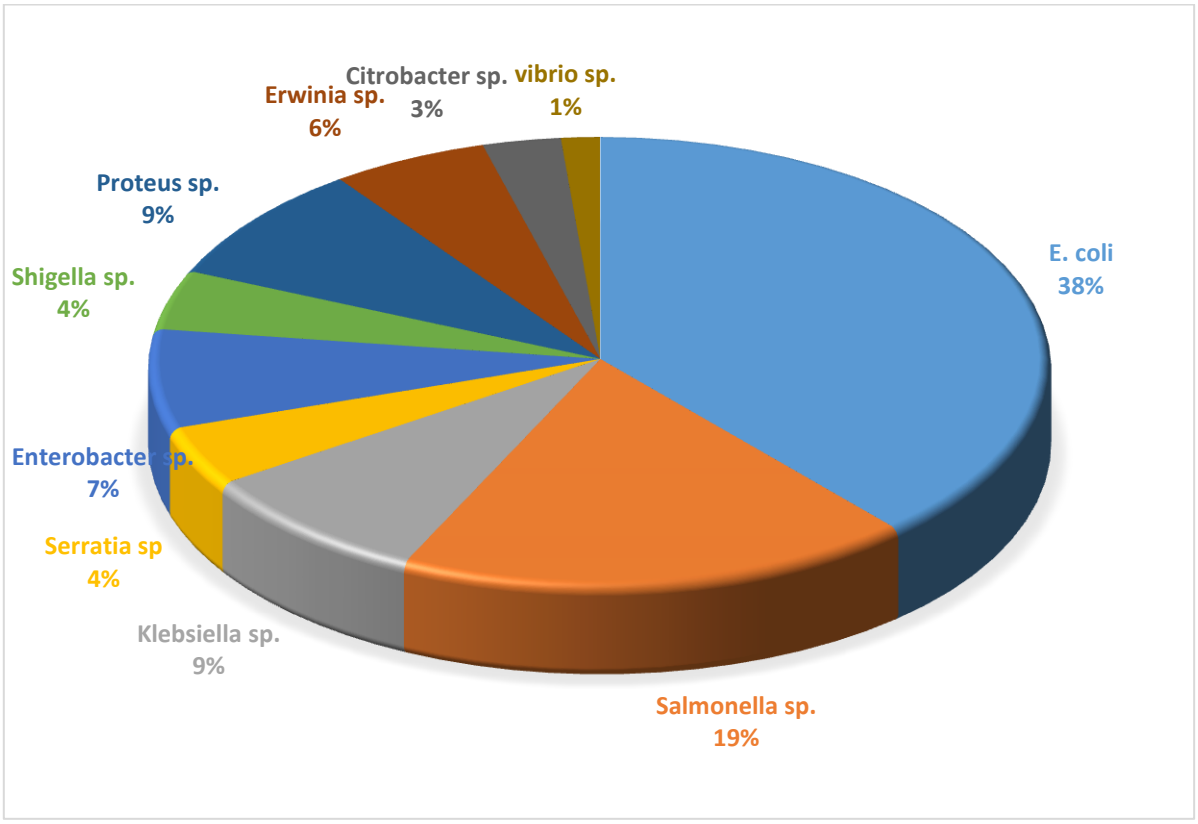

Figure 2: Percentages of the bacterial species isolated from Thiba River