# The bacterial flora of *Oreochromis niloticus* and *Clarias gariepinus* from earthen ponds in Sagana and Masinga, Kenya

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**Abstract.** *Karimi RD, Ngeranwa JJN, Njagi ENM, Kariuki S. 2022.* The bacterial flora of Oreochromis niloticus and Clarias gariepinus from earthen ponds in Sagana and Masinga, Kenya. *Intl J Bonorowo Wetlands 12: 63-73.* Food-borne diseases traced to fish consumption have been reported globally, including in Kenya. The aspect of food quality as far as fish consumption is concerned is underestimated in Kenya though aquaculture has been promoted. The bacterial flora of Tilapia (*Oreochromis niloticus* Linnaeus, 1758) and Catfish (*Clarias gariepinus* Burchell, 1822) from Masinga Dam and earthen ponds at Sagana fish farm was determined in this study to determine the anti-microbial response of the pathogenic bacteria. Tilapia fish and Catfish samples were collected from Masinga Dam and Sagana farm in the dry and rainy seasons. The fish were skinned, and gut content was taken for laboratory tests. The water and water sediment samples from these two study sites were also collected. Those samples were processed and cultured in MacConkey agar, and the selective media were subcultured in the colonies and then subjected to morphological examination from cultures. Then, the biochemical tests were carried out using commercially available API kits. The study showed the presence of bacterial species belonging to *Enterobacter* spp. (n=34), *Pseudomonas* spp. (n=6), *Aeromonas* spp. (n=5), *Vibrio* spp. (n=3) and *Acinetobacter* spp. (n=2) isolates during the dry season, while bacterial species belonging to *Enterobacter* spp. (n=31), *Pseudomonas* spp. (n=6), *Aeromonas* spp. (n=4) isolates during the dry season. The anti-microbial susceptibility analysis showed that the highest resistance rates were found against Ampicillin (Amp) (61.5% of isolates), Amoxicillin (AmL) (65.9% of isolates), Tetracycline (Te) (31.8% of isolates), and Chloramphenicol (C) (27.5% of isolates) while the lowest was Nalidixic acid (Na), Cefuroxime (Cxm) and Streptomycin (S) at (4.4% of isolates) each. All isolates were sensitive to Gentamycin (Gen), Ciprofloxacin (Cip), and Cefotaxime (CTX). The presence of the above organisms, some potentially pathogenic to humans, indicates that improperly handled, undercooked, or consumed raw fish may cause disease in susceptible individuals. At the same time, some isolates' anti-microbial resistance indicates that the use of antibiotics in aquaculture to promote growth should be studied further with a view to policy formulation.

**Keywords**: Bacteria, consumption, fish, microorganisms

## INTRODUCTION

Fish is an important component of diets worldwide, and an estimated 1 billion people rely on fish as their main source of animal protein (FAO 2007; Novoslavskij et al. 2016; Novoslavskij et al. 2016; Priatni et al. 2018; Aboagye et al. 2020). Fish production is estimated globally to be 148.5 million tonnes per year, which capture fisheries accounting for 88.6 million tonnes and aquaculture 59.9 million tonnes annually (FAO 2012). Fish at affordable prices provide much-needed protein to people, especially in developing countries. It sustains many through employment in fish and fish products-related services and feeds millions daily. In addition, their nutritional attributes are highly praised as it has high-quality vitamins, rich in essential amino acids, and their fatty acid fraction has well-established health benefits (with their anti-thrombotic activity) (Rahmanifarah et al. 2014; Partasasmita et al. 2015; Suvitha et al. 2015; Sayuti et al. 2022). Therefore, its availability in many developing countries should enable fish to contribute significantly to a balanced and healthy diet. Moreover, it is estimated that fish supplied around 60% of the population in many developing countries derives over 30% of their animal protein. In comparison, in most developed countries, almost 80% of the population obtains their animal protein supplies from fish, in less than 20% (FAO 2000).

The risks of food-borne disease associated with products from aquaculture are related to coastal or inland ecosystems, and the potential for environmental contamination is greater than in capture fisheries (Costa 2013). Most of the food safety hazards associated with products from aquaculture could be controlled by appropriate consumer education and good fish farm management practices concerning such risks as eating raw or partially cooked products that could contain pathogenic bacteria (Reilly and Kaferstein 1998). In developing countries, the estimated mortality of food and water-borne infectious diseases annually amounts to high death rates for children and infants. Moreover, microbiological food-borne illnesses affect up to 30% of the population in industrialized countries. It is estimated that each year 20 out of a million inhabitants die from food-borne diseases. Unwholesome fishery products and fish cause up to 30% of food-borne illnesses (WHO 1999).

In Asia, around 40 million people are affected by fish and water-borne parasitic diseases, especially trematodes. These parasitic diseases are widespread mainly in Viet Nam, Thailand, China, and Laos, where they encourage the consumption of raw fish as their habit. In addition, fish-borne illnesses can have costly health adverse effects on the economic losses incurred because of fish spoilage, medical expenses, the loss of productivity, and adverse publicity to the companies. Additional costs in international trade include the cost of rejections, recalls, product detections, and the resulting adverse publicity to the industry and even to the fish's country of origin (Lahsen 2003). Moreover, the financial implications of food-borne disease outbreaks could have grave consequences. An outbreak of cholera in Peru in 1991 cost 770 million dollars, for example, and a similar outbreak in Tanzania in 1998, cost 36 million dollars. However, effective surveillance systems and simple preventive measures, which would cost less, might have prevented these outbreaks and definitely reduced the impact (Lahsen 2003). For example, due to an outbreak of *Salmonella* in 1996 and an outbreak of cholera in 1997 in Kenya, there was a ban on fish and fishery exports to the EU (Ministry of Fisheries Development 1999).

Current knowledge of the impact of antibiotics used in aquaculture on the health and environment is poor, particularly in developing countries. In addition, drug residues may remain in fish used for human consumption; therefore, the antibiotics released into the environment can lead to antibiotic-resistant bacteria development in the food chain (Cabello 2006). Resistance to antibacterial agents is a global public health problem and one that is increasing as the antibacterial continues to lose effectiveness (Akinbowale et al. 2006).

This study aims to determine the bacterial flora of Tilapia, Catfish, water, and water sediments and their anti-microbial response from Masinga Dam and earthen ponds at Sagana fish farm during dry and wet seasons. Then, the specific objectives of this study are (i) To isolate and identify bacterial flora species during the dry and wet seasons at Masinga Dam and Sagana fish ponds, (ii) To identify whether there is a significant difference in the number of bacteria flora species isolated in fish specimen types from dams and ponds, during the dry and wet season, (iii) To determine the anti-microbial response of the pathogenic bacteria isolates from Masinga Dams and Sagana fish farm.

## MATERIALS AND METHODS

### Study area

The study was conducted at Masinga Dam and Sagana Aquaculture Centre, situated in the Tana River and Kirinyaga Districts, respectively. Masinga Dam is situated on the Tana River, and the main catches are Catfish and Tilapia. Dam fisheries account for about Kshs.34 million annually, with Nairobi City being the most consumed. Sagana Aquaculture Centre (Figure 1) is a Department of Fisheries breeding farm in Sagana town, Kirinyaga District (100 km North East of Nairobi, latitude 0°39'S and longitude 37°12'E, and altitude 1,230 m), with an area covering approximately 50 hectares, with ponds covering 18 hectares; each pond covers an area on average about 40 by 20 m$^2$ with a depth of 1 m. Ragati river water was diverted and delivered through a canal by gravity. It acts as a training center for fish farmers in aquaculture and one of the fisheries department's two main national fish hatcheries and acts as a training center for fish farmers in aquaculture.
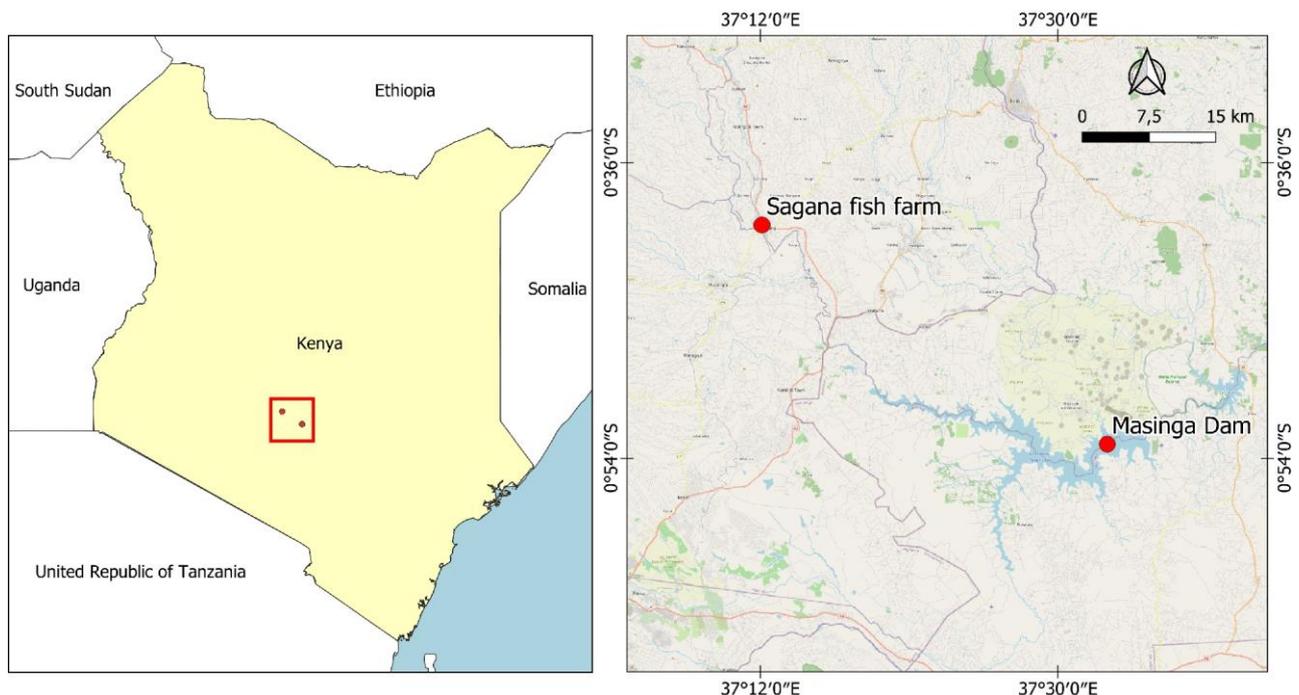


**Figure 1.** Map of Kenya showing Sagana fish farm and Masinga Dam, Kenya

The farm is involved in the culture of Catfish and Tilapia as the main species. In addition, the farm provides quality fish feeds to farmers, demonstrates the economic viability of integrated fish farming, and conducts research. Fish farming is conducted on still-water earthen ponds with semi-intensive systems. The ponds are fertilized using organic manure from cattle, chicken, and kitchen wastes and artificial fertilizers to enhance the growth of the phytoplankton. The other alternative feeds are wheat germ, rice bran, and maize bran. The experimental period started during the dry season in August 2007 and continued to December 2007 rainy season.

## Sample size

The sample size was determined using the ICMSF sampling standard, which relates the sampling plan stringency to the food's degree of hazard. When the health hazard is low, the A 3-class plan is used. In this plan, n = 5 and c = 3, n is the number of samples drawn, and the maximum allowable number of positive results is c (3).

## Sampling procedure

### Collection of fish samples

The study design adopted was a purposive study. Fish was sampled from the Sagana fish farm of 20 organically fertilized ponds; the ponds were selected at an interval of 4 ponds, and 5 pieces of table size Tilapia were harvested using a scoop net and taken in a cool box to the laboratory. The fish were aseptically skinned in the laboratory to get the skin sample. First, up to 25 g of fish skin was weighed and mixed with 225 mL of buffered peptone water in a sterile blender. Then, it was blended, and a part of the pure mix was inoculated into the MacConkey agar culture media. Next, to have distinguishable colonies, diluted subcultures were made from the initial culture after overnight incubation at $37^0$C at dilutions of 1:10, 1:100, and 1:1,000 using peptone water and incubated overnight at $37^0$C. Growth of distinct colonies was achieved at the 1:100 dilutions. Next, the colonies were sub-cultured in various selective media Hektoen Enteric (HE) agar, Xylose-Lysine Deoxycholate (XLD) agar, and *Salmonella-Shigella* (SS) agar and incubated. The colonies were morphologically identified and, for further identification, subjected to biochemical tests. Final identification was done using the API 20E method.

The fish was cleaned using 70% alcohol for the gut sample, an incision was made over the peritoneal cavity, and dissected to get the gut contents. The gut contents were combined and weighed, similarly to the skin, and the same procedure was followed, and again, the growth of distinct colonies was at 1:100 dilutions. These same procedures were conducted for the dam fish during the two seasons.

### Collection of water samples

Water was collected from one end of the pond and the center, about 20 cm beneath the surface, in sterile bottles of 100 mL. The water sample was incubated at $37^0$C overnight and enriched using peptone water; this was inoculated to MacConkey culture media and incubated at $37^0$C overnight. The growth of distinct colonies was obtained at the 1: 10 dilution. Then, they were sub-cultured in selective media and incubated. The colonies were morphologically identified and then subjected to biochemical tests for further identification. The final identification was using the API 20E method.

The Dam water was collected in the early morning and taken within 2 hours to the laboratory. The sample was collected using sterile bottles away from the bank at a one-foot depth below the surface, and the bottle mouth was directed towards the current, filled, and covered immediately. The dam water was stored in a cooler box during transportation. The same procedure was used for the pond water applied to the dam water.

### Collection of water sediments

Sediment samples were collected from the bottom of the dam and pond using an Ekman grab in all procedures, with the sediments collected at the edge and the center of the ponds. The sediments were then taken to the laboratory, mixed, and enriched using peptone water cultured in MacConkey and incubated at $37^0$C overnight. Next, the colonies were sub-cultured in selective media. The colonies were morphologically identified and then subjected to biochemical tests, with the final identification using the API 20E method.

## The Analytical Profile Index (API 20E)

All test chambers were rehydrated by inoculation with a saline suspension of a pure culture of the bacterial strain subjected to the identification. After incubation in a humidity chamber at 37°C for 18 to 24 hours, the color reactions were read. The test reaction results were converted to a seven-digit code. Then, the code was looked up in the database book for the genus and species identification of the test microorganism.

## Antibiotic susceptibility testing

### Antibiotic sensitivity profile

The antibiotic sensitivity testing against commonly used anti-microbial agents' was performed on all the isolates (Table 1). The Kirby – Bauer disk diffusion test was the standard recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2009). After incubation at $37^0$C for 24 hours, the diameters of inhibition zones were measured and compared with the control organism *Escherichia coli* the ATCC 25922.

**Table 1.** Anti-microbial agents used for sensitivity testing

| Anti-microbial agent | Concentration |
|---|---|
| Ampicillin (Amp) | 10 µg/mL |
| Chloramphenicol (C) | 30 µg/mL |
| Streptomycin (S) | 10 µg/mL |
| Tetracycline (Te) | 30 µg/mL |
| Nalidixic acid (Na) | 30 µg/mL |
| Ciprofloxacin (Cip) | 5 µg/mL |
| Gentamycin (Gen) | 10 µg/mL |
| Cefuroxime (Cxm) | 30 µg/mL |
| Amoxicillin (Aml) | 5 µg/mL |
| Cefotaxime (CTX) | 30 µg/mL |

## RESULTS AND DISCUSSION

**Bacteria isolates per specimen type**

The sum of 91 bacteria were isolates from six specimen types: Tilapia Gut, Tilapia skin, Catfish skin, Catfish Gut, and water and water sediments. The Tilapia gut had the highest proportion (20; 22%) of bacteria isolates than the rest. Tilapia skin and catfish skin isolates at 16 (17.6%) bacteria. The water and catfish guts had the same number of bacteria isolates at 15 (16.5%). The water sediments had the lowest proportion (9; 9.9%) of bacteria isolates compared to the rest of the specimens (Figure 2).

**Types of bacteria isolates from Catfish, Tilapia, water, and water sediments**

A total of 91 (100%) bacterial isolates were identified, of which *Citrobacter freundii* were 16 (17.6%) than the rest of the bacteria isolates. The *E. coli* and *C. freundii* are the only bacteria that occurred in five specimen types except in water sediments and Tilapia skin, respectively (Table 2).

In Catfish skin, the bacterial isolates were 16 (17.6%) same as the bacterial isolates in Tilapia skin. The bacteria isolates that occurred both in Tilapia Skin and Catfish skin were: *Aeromonas sobia, C. freundii, E. coli, Edwardsiella tarda, Enterobacter cloacae, Enterobacter sakazakii, Proteus mirabilis* and *Pseudomonas aeruginosa* (Table 2). In Tilapia Gut, 20 (22.0%) bacterial isolates were identified. On the contrary, 15 (16.5%) bacteria were isolates from Catfish Gut. Bacterial isolates that occurred in both Tilapia gut and Catfish skin were; *A. sobia, C. freundii, E. tarda, E. sakazakii, E. coli,* and *Pseudomonas*

*fluorescens* (Table 2). In water, 15 (16.5%) bacteria were isolates, then 9 (9.9%) bacterial isolates from water sediments. Bacterial isolates in water and water sediments were; *Enterobacter fergusonii, E. coli, P. fluorescens,* and *Salmonella* spp. (Table 2).

**Types of bacteria isolates from Sagana Pond and Masinga Dam, Kenya**

The bacteria isolates from Sagana Ponds were 54 (59.3%), while from Masinga Dam were 37 (40.7%) isolates. The *C. freundii* found 16 (17.6%), of which 10 (11.0%) were isolates from Sagana Pond, while from Masinga Dam, were 6 (6.6%). All bacteria isolates include *A. sobia, C. freundii, E. coli, E. tarda, P. aeruginosa, P. fluorescens,* and *P. mirabilis,* occurred in both Masinga Dam and Sagana Pond (Table 3).



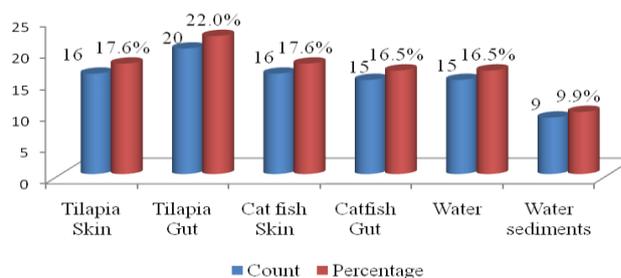**Figure 2.** Bacteria isolates from various collected samples

**Table 2.** Bacteria isolates in Tilapia, Catfish, water, and water sediments

| Isolates | Tilapia skin | Catfish skin | Tilapia gut | Catfish gut | Water | Water sediments | Total |
|---|---|---|---|---|---|---|---|
| *Acinetobacter* spp | - | - | - | - | 1 | - | 1 (1.1%) |
| *Aeromonas sobia* | 2 | 2 | 1 | 1 | - | - | 6 (6.6%) |
| *Chromobacterium violaceum* | - | - | 1 | - | - | - | 1 (1.1%) |
| *Citrobacter freundii* | 4 | 2 | 5 | 3 | 2 | - | 16 (17.6%) |
| *Escherichia coli* | - | 1 | 2 | 2 | 5 | 4 | 14 (15.4%) |
| *Edwardsiella tarda* | 1 | 1 | 2 | 2 | - | - | 6 (6.6%) |
| *Enterobacter agglomerans* | - | 1 | - | - | - | 1 | 2 (2.2%) |
| *Enterobacter amnigenus* | - | - | 1 | - | - | - | 1 (1.1%) |
| *Enterobacter cloacae* | 1 | 1 | - | - | 1 | - | 3 (3.3%) |
| *Enterobacter fergusonii* | - | - | - | - | 1 | 1 | 2 (2.2%) |
| *Enterobacter sakazakii* | 1 | 1 | 1 | 3 | - | - | 6 (6.6%) |
| *Klebsiella onithnolytica* | 1 | - | - | - | - | - | 1 (1.1%) |
| *Klebsiella pneumonia* | 1 | - | 1 | - | - | - | 2 (2.2%) |
| *Plesiomonas shigelloides* | - | - | - | - | 1 | - | 1 (1.1%) |
| *Proteus mirabilis* | 1 | 1 | - | - | - | 1 | 3(3.3%) |
| *Providentia stuartii* | - | 3 | - | 2 | - | - | 5 (5.5%) |
| *Pseudomonas aeruginosa* | 2 | 3 | 3 | - | 1 | - | 9 (9.9%) |
| *Pseudomonas fluorescens* | - | - | 1 | 1 | 1 | 1 | 4 (4.4%) |
| *Salmonella spp* | 1 | - | 1 | - | 1 | 1 | 4 (4.4%) |
| *Shigella boydii* | - | - | - | 1 | - | - | 1 (1.1%) |
| *Vibrio mechnikovii* | 1 | - | - | - | 1 | - | 2 (2.2%) |
| *Vibrio vulnificus* | - | - | 1 | - | - | - | 1 (1.1%) |
| Total | 16 (17.6%) | 16 (17.6%) | 20 (22.0%) | 15 (16.5%) | 15 (16.5%) | 9 (9.9%) | 91 (100.0%) |

**Table 3.** Types of bacteria isolated from Sagana Pond and Masinga Dam, Kenya

| Bacteria isolated | Sagana Pond | Masinga Dam | Total |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| *Acinetobacter* spp | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Aeromonas sobia** | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| *Chromobacterium violaceum* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Citrobacter freundii** | 10 (11.0) | 6 (6.6) | 16 (17.6) |
| *Escherichia coli** | 6 (6.6) | 8 (8.8) | 14 (15.4) |
| *Edwardsiella tarda** | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| *Enterobacter aggromerans* | 0 (0.0) | 2 (2.2) | 2 (2.2) |
| *Enterobacter amnigenus* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Enterobacter fergusoni* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Enterobacter sakazakii* | 0 (0.0) | 6 (6.6) | 6 (6.6) |
| *Enterobacter cloace* | 0 (0.0) | 3 (3.3) | 3 (3.3) |
| *Klebsiella onithnolytica* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Klebsiella pneumonia* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Plesiomonas shigelloides* | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| *Proteus mirabilis** | 1 (1.1) | 2 (2.2) | 3 (3.3) |
| *Providencia stuartii* | 5 (5.5) | 0 (0.0) | 5 (5.5) |
| *Pseudomonas aeruginosa** | 7 (7.7) | 2 (2.2) | 9 (9.9) |
| *Pseudomonas fluorescens** | 2 (2.2) | 2 (2.2) | 4 (4.4) |
| *Salmonella* spp | 4 (4.4) | 0 (0.0) | 4 (4.4) |
| *Shigella boydii* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Vibrio mechnikovii* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Vibrio vulnificus* | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| Total | 54 (59.3) | 37 (40.7) | 91 (100) |

Note: + /- Species occurred/ did not occur, * Bacteria isolated both in Sagana and Masinga Dam, Kenya

### Bacteria isolates from Sagana Pond and Masinga Dam by specimen type

Tilapia gut had a total of 20 (22.0%) bacteria isolates, of which 12 (13.2%) were from Sagana Ponds and Masinga were 8 (8.8%) bacteria isolates. Water sediments had the lowest proportion (9; 9.9%) from Sagana Ponds (4; 4.4%) and Masinga Dam (5; 5.5%) of bacteria isolates, respectively. When the results were subjected to chi-square, there was no significant difference between a pond and dam water in bacteria flora species isolates, $x^2 = 3.853$, $df=5$, =0.571) (Table 4).

### Bacteria isolates from Tilapia, Catfish, water, and water sediments in dry and wet seasons

The tilapia gut bacteria isolates were (20; 22%), of which 11 (12.1%) were isolates during dry seasons, while in the wet season were 9 (9.9%) bacteria isolates. In all specimen types, there were more bacteria isolates during the dry season than the wet season, except the tilapia skin specimen, where (6; 6.6%) bacteria were isolates in the dry

and 10 (11.0%) in the wet season, respectively. The reason adduced for a higher number of bacteria isolates during the dry season than the wet season, according to Wemedo (2002), is that lower temperatures inhibited microbial activity during the wet season. Another reason for this phenomenon is that the soil saturation by rain reduces aeration by limiting activity (Marshall and Devinny 1998). (Figure 3).

### Types of bacteria isolate from the dry and wet season

During the dry season, 50 (54.9%) bacteria were isolates, while 41 (45.1%) bacteria were isolates in the wet season. The number of *C. freundii* isolates from both dry and wet season were 16 (17.6%), of which 9 (9.9%) were isolates during the dry season while 7 (7.7%) were isolates in the wet season, *A. sobia, Acinetobacter* spp., *C. freundii, E. sakazakii, E. tarda, E. coli, E. cloacae, P. aeruginosa,* and *Providencia stuartii* were isolates in both seasons (Table 5).

### Bacteria isolates during the wet and dry seasons by specimen type

The total of all bacterial isolates was 91, with tilapia fish gut having 20 (22.0%), of which 11 (12.1%) were during the dry season while 9 (9.9%) were during the wet season. There was no significant difference between the number of bacteria isolates from the Tilapia fish gut and the two seasons (p>0.05). That was the same for other specimen types despite more bacteria isolates during the dry season (Table 6), including Tilapia fish skin which had 6 (6.6%) and 10 (11.0%) bacteria isolates from both dry and wet seasons, respectively, There was no significant difference to show any association of bacteria isolates and the source ( $x^2 = 3.006$, *df*=5, P=0.699).
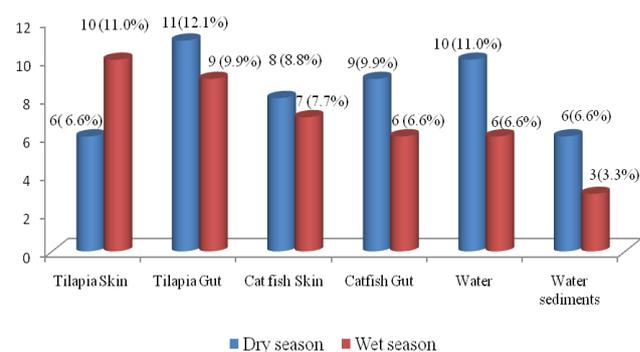


**Figure 3.** Bacteria isolates in the dry and wet seasons by specimen type

**Table 4.** Number of bacteria isolated from Sagana farm and Masinga Dam, Kenya

| Specimen type | Sagana Farm n (%) | Masinga Dam n (%) | Total n (%) | $x^2$ | *Df* | P-Value |
|---|---|---|---|---|---|---|
| Tilapia Skin | 12 (23.2%) | 4 (4.4%) | 16 (17.6%) | 4.00 | 1 | 0.050 |
| Tilapia Gut | 12 (23.2%) | 8 (8.8%) | 20 (22.0%) | 0.800 | 1 | 0.371 |
| Catfish Skin | 9 (9.9%) | 7 (7.7%) | 16 (17.6%) | 0.250 | 1 | 0.617 |
| Catfish Gut | 7 (7.7%) | 8 (8.8%) | 15 (16.5%) | 0.670 | 1 | 0.796 |
| Water | 10 (11.0%) | 5 (5.5%) | 15 (16.5%) | 1.667 | 1 | 0.197 |
| Water sediments | 4 (4.4%) | 5 (5.5%) | 9 (9.9%) | 0.111 | 1 | 0.739 |
| Total | 54 (59.3%) | 37 (40.7%) | 91 (100%) | 3.853 | 5 | 0.571 |

Note: *:Significant at 0.05

**Table 5.** Bacteria isolates from the dry and wet season

| Bacteria isolates | Dry season n (%) | Wet season n (%) | Total n (%) |
|---|---|---|---|
| *Acinetobacter* spp.* | 1 (1.1) | 1 (1.1) | 1 (1.1) |
| *Aeromonas sobia** | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| *Chromobacterium iolaceum* | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| *Citrobacter freundii** | 9 (9.9) | 7 (7.7) | 16 (17.6) |
| *Escherichia coli** | 6 (6.6) | 8 (8.8) | 14 (15.4) |
| *Edwardsiella tarda** | 3 (3.3) | 3 (3.3) | 6 (6.6) |
| *Enterobacter aggromerans* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Enterobacter amnigenus* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Enterobacter fergusoni* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Enterobacter sakazakii** | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| *Enterobacter cloace** | 2 (2.2) | 1 (1.1) | 3 (3.3) |
| *Klebsiella onithnolytica* | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| *Klebsiella pneumonia* | 0 (0.0) | 2 (2.2) | 2 (2.2) |
| *Plesiomonas shigelloides* | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| *Proteus mirabilis* | 0 (0.0) | 3 (3.3) | 3 (3.3) |
| *Providencia stuartii** | 3 (3.3) | 2 (2.2) | 5 (5.5) |
| *Pseudomonas aeruginosa** | 4 (4.4) | 5 (5.5) | 9 (9.9) |
| *Pseudomonas fluorescens* | 0 (0.0) | 4 (4.4) | 4 (4.4) |
| *Salmonella* spp. | 4 (4.4) | 0 (0.0) | 4 (4.4) |
| *Shigella boydii* | 1(1.1) | 0 (0.0) | 1(1.1) |
| *Vibrio mechnikovii* | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| *Vibrio vulnificus* | 1(1.1) | 0 (0.0) | 1(1.1) |
| Total | 50 (54.9) | 41 (45.1) | 91(100) |

Note: +: Species occurred, -: Species did not occur, *: Bacteria isolated in both dry and wet season

**Table 6.** Bacteria isolates in specimen type by season

| Specimen type | Dry Season | Wet Season | Total | X² | df | P-value |
|---|---|---|---|---|---|---|
| Tilapia Skin | 6 (6.6%) | 10 (11.0%) | 16(17.6%) | 1.000 | 1 | 0.317 |
| Tilapia Gut | 11(12.1%) | 9(9.9%) | 20(22.0%) | 0.200 | 1 | 0.655 |
| Catfish Skin | 8 (8.8%) | 7(7.7%) | 15(16.5%) | 0.067 | 1 | 0.796 |
| Catfish Gut | 9 (9.9%) | 6(6.6%) | 15(16.5%) | 0.600 | 1 | 0.439 |
| Water | 10 (11.0%) | 6(6.6%) | 16(17.6%) | 1.000 | 1 | 0.317 |
| Water sediment | 6 (6.6%) | 3(3.3%) | 9(9.9%) | 1.000 | 1 | 0.317 |
| Total | 50 (54.9%) | 41(45.05%) | 91(100%) | 3.006 | 5 | 0.699 |

## Correlation between bacteria isolates from specimen types, sites, and season

There was a strong positive correlation between bacteria isolates from the specimen and the site during the dry and wet seasons (Ponds and Dams). Furthermore, from the two sites, the more bacteria isolates, the higher the significant difference between the bacteria isolated during dry and wet seasons (r=0.734, P=0.000) (Table 7). On the other hand, Pearson's correlation analysis did not indicate a significant correlation between the bacteria isolates from the specimen and the site. The correlation between the two variables was weak and negatively correlated (r=0.734, P=0.000) (Table 7). On the other hand, between the bacteria isolates from specimen types and the site, there was a weak positive correlation(Sagana Ponds and Masinga Dam) (r=0.136, P=0.197) (Table 7).

## Overall antibacterial response of isolates

All bacteria isolates were examined to commonly used anti-microbial agents for susceptibility. The inhibition

zones were read after incubation, compared against measurement standards, and recorded as resistant, intermediate, or sensitive. The CIP antibiotic was susceptible to all (100%) bacterial isolates; on the contrary, none of the bacterial isolates registered resistance to GEN, CXT, and CIP antibiotics. The drug with the highest resistance was AML, with 60 (65.9%) bacteria isolates registering resistance, then by AMP at 56 (61.5%) bacterial isolates. On average, 64 (70.3%) of bacteria isolated registered susceptibility, 18 (20%) registered resistance to drugs, and 9 (9.6%) registered intermediate (Table 8 and Figure 4).

## Antibacterial response of isolates from Sagana Ponds

The total Sagana Ponds isolates were 54, with 38 (70.4%) registered resistance to 7 anti-microbial agents. Bacterial isolates from Sagana Ponds registered high resistance to AML and AMP drugs, 38 (70%) and 36 (67%), respectively. The rest of the isolates' resistance registered to antibiotics was; 16 (30%) were resistant to TE, 15 (28%) to C, and 5 (10%) to NA and S, respectively. No resistance was registered to CIP, CXT, and GEN for bacterial isolates from Sagana Ponds (Figure 5)

Concerning bacteria type isolates from Sagana Pond, *Salmonella* spp. showed resistance to AMP, AML, and CXM. Most bacterial isolates from Sagana showed resistance to an average of 2 to 3 drugs except for klebsiella pneumonia, which registered resistance to five antibiotics: AML, AMP, TE, S, and C. There was variation in the antibacterial response of isolates from the Sagana Pond (F=8.4, P=0.000) (Table 9).

**Table 7.** correlation between specimen, site, and season

| | | Season | Site |
|---|---|---|---|
| **Site** | Pearson Correlation | 0.734** | 1 |
| | Sig. (2-tailed) | **0.000** | |
| | N | 91 | 91 |
| **Specimen** | Pearson Correlation | -0.162 | 0.136 |
| | Sig. (2-tailed) | 0.124 | 0.197 |
| | N | | 91 |

Note: **: Correlation is significant at the 0.01 level (2-tailed)

**Table 8.** Antimicrobial response of isolates to various antibiotics

| Antibiotic | Resistant N | Resistant % | Sensitive n | Sensitive % | Intermediate n | Intermediate % | Total N | Total % |
|---|---|---|---|---|---|---|---|---|
| AML | 60 | 65.9 | 27 | 29.6 | 4 | 5.5 | 91 | 100 |
| CXT | 0 | 0 | 83 | 91.2 | 8 | 7.14 | 91 | 100 |
| CIP | 0 | 0 | 91 | 100 | 0 | 0 | 91 | 100 |
| S | 4 | 4.4 | 69 | 75.8 | 18 | 19.7 | 91 | 100 |
| AMP | 56 | 61.5 | 29 | 31.8 | 6 | 6.6 | 91 | 100 |
| GEN | 0 | 0 | 90 | 98.9 | 1 | 1.1 | 91 | 100 |
| TE | 29 | 31.8 | 40 | 43.9 | 22 | 24.2 | 91 | 100 |
| C | 25 | 27.5 | 47 | 51.6 | 19 | 20.9 | 91 | 100 |
| NA | 4 | 4.4 | 78 | 85.7 | 9 | 9.8 | 91 | 100 |
| CXM | 4 | 4.4 | 86 | 94.5 | 1 | 1.1 | 91 | 100 |
| Average | 18 | 20.0 | 64.0 | 70.3 | 9 | 9.6 | 91 | 100 |
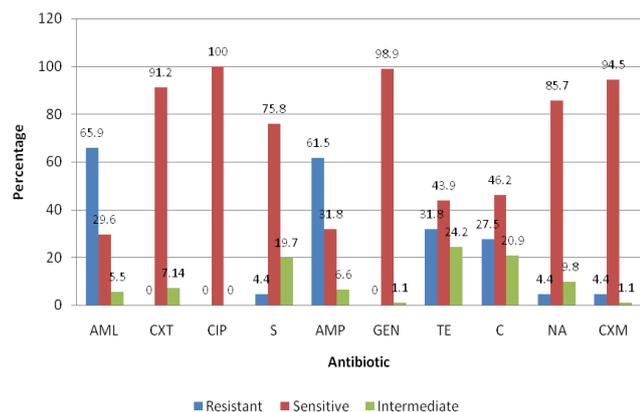
**Figure 4.** Percentage distribution of anti-microbial response of isolates to various antibiotics
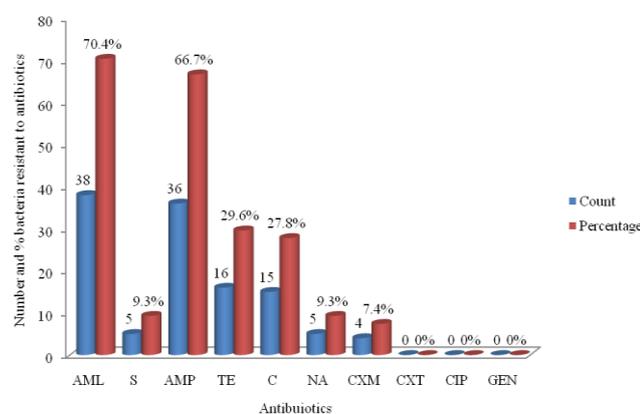


**Figure 5.** Number of Isolates resistant to antibiotics from Sagana, Kenya
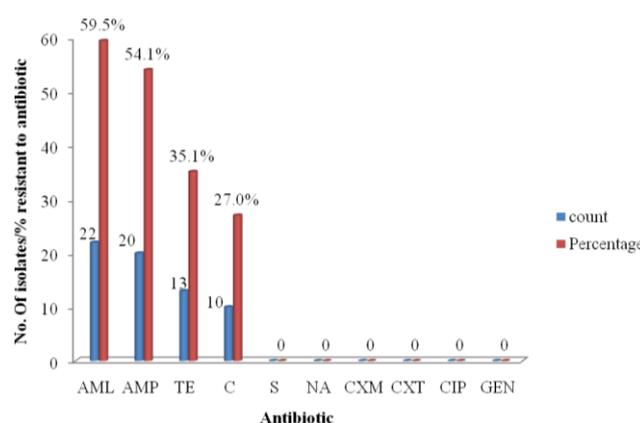


**Figure 6.** Number of bacterial isolates from Masinga Dam, Kenya, resistant to antibiotics

**Antibacterial response of Isolates from Masinga Dam**

Bacterial isolates from Masinga Dam registered resistance to four antibiotics: AML, AMP, TE, and C. There were 37 isolates, with 22 (60%) isolates registering displayed high resistance in AML, followed by 20 (54%) bacteria that displayed resistance to AMP. Of the rest, 13 (35%) registered resistance to Te, and 10 (27%) displayed resistance to C (Figure 6).

The bacterial isolates from Masinga Dam registered resistance to at least one antibiotic, *Pleisiomonas shigelloides* registered resistance to AML and AMP, while *E. coli,* to AML, AMP, and TE. Like the isolates from the Sagana Pond, the antibacterial response of isolates from the Masinga Dam was more resistant to AML and AMP than the rest of the antibiotics. However, for bacterial isolates from the Masinga, there was no significant difference in an antibacterial response for bacterial isolates from the dam (F=1.84, P=0.14) (Table 10).

**Discussion**

Members of Enterobacteriaceae are part of the gut flora found in the intestines of humans and other animals. In contrast, others are found in soil, water, or parasites on various animals and plants. Although in this study, there is no exception for fish, most of the bacteria isolates were from the family Enterobacteriaceae; *C. freundii, E. sakazakii, E. cloacae, E. tarda, Enterobacter amnigenus, Enterobacter agglomerans*, *E. coli, Klebsiella ornithinolytica*, *Klebsiella pneumoniae, P. mirabilis, P. stuartii, Shigella boydii, Salmonella* spp., and *P. shigelloides*. That aligns with the findings of Ogbondeminu and Olayemi (1993), who reported that 50% of fish and water of the microorganisms recovered from an earthen pond fertilized with animal fecal waste were members of the family Enterobacteriaceae.

The other bacteria isolated include; *A. sobia, Chromobacterium violaceum, P. aeruginosa, P. fluorescens, Vibrio mechnikovii,* and *Acinetobacter* spp.. They are widely distributed in the marine environment and the soil and have been implicated in causing human diseases as opportunistic pathogens. In other studies by Nganou et al. (2011), these bacteria have been isolates from tilapia fish, which isolate *Aeromonas* spp.*, Vibrio* spp.*, Pleisomnas* spp.*, Acinetobacter* spp.*,* Enterobacteriaceae*, Pseudomonas* spp.*,* collected from four lakes in Cameroon. Other studies (Naim et al. 2012) recovered A. *hydrophila, Edwardsiella* spp., *Streptococcus* spp., *S. putrefaciens, Staphylococcus* sp., and *Vibrio* spp. In addition, *Aeromonas* spp.*, C. freundii, C. violaceum, E. coli,* and *P. shigelloides* were isolates in the gastrointestinal regions of semi-intensively cultured Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). Although these bacteria are not often associated with fish or enteric diseases in human beings, the health implications should not be ignored on introducing these organisms into natural water via the fish feces in aquaculture wastewaters (Naim et al. 2012).

During the two seasons, more bacterial species isolates from the ponds than from the dams. Furthermore, there was a correlation between the sites where the bacteria were isolates, which could be attributed that the ponds being fertilized with animal manure to enhance alga bloom and the accumulation of fish feces and leftover feed in the earthen pond (Davis and Goulder 1993; Makosora and Jazek 1994). In addition, according to Wemedo (2002), higher temperatures inhibit micro-bacterial activity, which supports more bacteria isolates during the dry season compared to the wet season. Another reason for this phenomenon is that soil saturation by rain limits activity by reducing aeration (Marshall and Devinny 1998).

**Table 9**. Antimicrobial response of isolates from Sagana, Kenya

| Isolates | No | AML | | | CTX | | | CIP | | | S | | | AMP | | | GEN | | | TE | | | C | | | NA | | | CXM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I |
| *Citrobacter freundii* | 10 | 9 | 1 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 5 | 5 | 9 | 1 | 0 | 0 | 10 | 0 | 2 | 4 | 4 | 3 | 4 | 3 | 0 | 10 | 0 | 0 | 10 | 0 |
| *Aeromonas sobia* | 4 | 4 | 3 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 6 | 1 | 6 | 1 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 |
| *Vibrio mechnikovii* | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Salmonella* spp. | 4 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Edwardsiella tarda* | 4 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Shigella boydii* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Escherichia coli* | 6 | 0 | 5 | 1 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 4 | 1 | 0 | 6 | 0 | 3 | 2 | 1 | 2 | 2 | 2 | 0 | 4 | 2 | 0 | 6 | 0 |
| *Enterobacter amnigenus* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Enterobacter fergusonii* | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Acinetobacter* spp. | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Pseudomonas aeruginosa* | 7 | 7 | 0 | 0 | 0 | 6 | 1 | 0 | 7 | 0 | 1 | 4 | 2 | 5 | 1 | 1 | 0 | 6 | 1 | 3 | 0 | 4 | 5 | 0 | 2 | 4 | 3 | 0 | 3 | 4 | 0 |
| *Klebsiella onithnolytica* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Chromobacterium violaceum* | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| *Proteus mirabilis* | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Pseudomonas flurosecenes* | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 |
| *Klebsiella pneumonia* | 2 | 2 | 2 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 1 | 3 | 0 | 2 | 2 | 0 | 0 | 4 | 0 | 2 | 1 | 1 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 |
| *Providencia stuartii* | 5 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 |
| Total | 54 | 34 | 15 | 2 | 0 | 52 | 2 | 0 | 54 | 0 | 2 | 40 | 11 | 34 | 16 | 4 | 0 | 51 | 2 | 14 | 26 | 14 | 16 | 26 | 9 | 6 | 44 | 5 | 3 | 48 | 2 |
| % | 100 | 63 | 28 | 4 | 0 | 97 | 4 | 0 | 100 | 0 | 4 | 74 | 20 | 63 | 30 | 7 | 0 | 94 | 4 | 26 | 48 | 26 | 30 | 48 | 17 | 11 | 82 | 9 | 6 | 89 | 4 |

Note: F=8.4, df=127, P-value=0.000

**Table 10.** Antibacterial response of isolates from Masinga Dam, Kenya

| Isolates | No | AML | | | CTX | | | CIP | | | S | | | AMP | | | GEN | | | TE | | | C | | | NA | | | CXM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I |
| *Citrobacter freundii* | 6 | 5 | 1 | 0 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 3 | 3 | 5 | 1 | 0 | 0 | 6 | 0 | 1 | 3 | 2 | 3 | 3 | 0 | 0 | 6 | 0 | 0 | 6 | 0 |
| *Aeromonas sobia* | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Vibrio vulnificus* | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Edwardsiella tarda* | 2 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Escherichia coli* | 8 | 2 | 6 | 0 | 0 | 6 | 2 | 0 | 5 | 3 | 0 | 6 | 2 | 2 | 3 | 3 | 0 | 6 | 2 | 2 | 4 | 2 | 0 | 4 | 4 | 0 | 4 | 4 | 0 | 8 | 0 |
| *Enterobacter sakazakazii* | 6 | 3 | 3 | 0 | 0 | 6 | 0 | 0 | 3 | 3 | 0 | 6 | 0 | 2 | 4 | 0 | 0 | 3 | 3 | 2 | 3 | 1 | 1 | 4 | 1 | 0 | 4 | 2 | 0 | 4 | 2 |
| *Enterobacter cloace* | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 |
| *Pseudomonas fluorescens* | 2 | 2 | 0 | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 3 | 0 | 1 | 0 | 6 | 1 | 3 | 0 | 4 | 4 | 1 | 2 | 0 | 3 | 4 | 0 | 4 | 0 |
| *Proteus mirabilis* | 2 | 2 | 2 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 2 | 1 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 |
| *Enterobacter aggromerans* | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 |
| *Plesiomonas shigelloides* | 2 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 |
| *Pseudomonas aureginosa* | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Total | 37 | 22 | 16 | 1 | 0 | 35 | 5 | 0 | 31 | 8 | 0 | 32 | 11 | 20 | 13 | 4 | 0 | 35 | 9 | 13 | 22 | 10 | 10 | 25 | 9 | 0 | 34 | 10 | 0 | 38 | 3 |
| % | 100 | 59 | 43 | 3 | 0 | 95 | 14 | 0 | 84 | 22 | 0 | 86 | 30 | 54 | 35 | 11 | 0 | 95 | 24 | 35 | 59 | 27 | 27 | 68 | 24 | 0 | 92 | 27 | 0 | 103 | 8 |

This study also revealed that all the specimens sampled sediment had the lowest number of bacteria isolates; this aligns with an earlier study by Niemi and Taipalinen (1982), who reported a lack of sunlight could play an important role in the bacteria growth that could be attributed to a very low count in sediment (Ferguson et al. 1996). In this study, there were more bacteria in all specimens during the dry season; this could be attributed to bacteria multiplying more in high temperatures. (Chowdhury et al. 2000) observed similar results in Tilapia's intestinal Tilapia bacterial load. Research by Sugita et al. (1985) and Markosova and Jezek (1994) reported that during summer months, populations of indicator bacteria increased with increasing water temperature as the temperature became favorable for the growth of bacteria.

The findings from the dams and the ponds revealed that different members of Enterobacteriaceae were isolates, some of which are pathogenic and others non-pathogenic. In other studies, there are reports of isolation of different members of Enterobacteriaceae as potential fish and human pathogens from naturally manured carps, Tilapia, eel, striped bass, and its earthen culture environment (Nair and Nair 1988; Nedoluha and Westhoff 1997; Muratori et al. 2000). This pathogenic Enterobacteriaceae was more in the Sagana Ponds than in the Masinga Dam, which could be due to the animal manure in the ponds (Ogbondeminu and Olayemi 1993). In the present study, *Salmonella* members of Enterobacteriaceae and *Shigella* have recovered from ponds at Sagana fish ponds, at fish, water, and water sediments, where they use integrated fish culture systems. That indication was observed in naturally manured earthen ponds by Nedoluha and Westhoff (1997) of an inherent risk of contamination by pathogens in the environment. The presence of Salmonella spp. indicates feacal contamination of water from which the fishes were harvested.

*Aeromonas sobria* was one of the bacteria isolates from both sources during the two seasons. It is a well-known human pathogen (Mateos et al. 1993; Thune et al. 1993, Austin and Adams 1996) and therefore poses a risk to consumers of fish-borne *Aeromonas* gastroenteritis if not properly cooked. Furthermore, the finding of *vibrio* spp. during the dry season aligns with the studies conducted by (Al-Harbi and Uddin 2003), who found more bacterial counts during the summer than in winter.

The *P. aeruginosa,* a potential human pathogen that can persist even after processing, was isolates during the two seasons, posing a health hazard to consumers. Furthermore, Lyhs et al. (1998) reported that *Pseudomonas* was the organism important in food spoilage, with economic losses responsible for 15.3% of spoilage of preserved fish products. Therefore, the fish from both the Sagana Ponds and the Masinga Dam should be processed and stored properly to eliminate contamination.

The *E. coli* has been recognized traditionally as an indicator organism of fecal contamination of seafood and water (Geldreich 1997). The *E. coli* are inhabitants normally of the intestinal tracts of all warm-blooded animals. In this study, *E. coli* was recovered in all fish and water samples indicating poor sanitary condition and hygiene in Sagana Ponds and Masinga Dams. Similar to many landing beaches in Kenya, the lack of proper sanitation facilities at the Masinga Dam landing sites could explain the presence of *E. coli* in all specimens. According to Chandraval et al. (2010), other studies found that fish and water samples were contaminated with fecal coliforms like *E. coli* from the Nadia District of West Bengal in India.

The *E. tarda* isolates from fish samples in both seasons are considered a serious problem in subtropical or tropical areas. Infections associated with this species include wound infections, gastroenteritis, and systemic diseases such as meningitis, cholecystitis, septicemia, and osteomyelitis (Janda and Abbott 1993). In addition, *E. tarda* has been isolates in fish from retail markets and freshwater aquaculture environments in India (Pankajkumar 2009).

The *P. mirabilis* and *C. freundii* were isolates in the Masinga Dam samples and have also been isolates in other studies (Niemi and Taipalinen 1982; Apun et al. 1999).

The *C. violaceum* is a Gram-negative rod isolated from soil and water in subtropical and tropical regions, while in this study, it was isolates in Sagana Ponds only. Even though infections caused by *C. violaceum* are rare among mammals, Apun et al. (1999) reported two cases of human infection caused by both pigmented and non-pigmented strains of *C. violaceum*. The *P. shigelloides* is a common pathogen in tropical regions associated with occasional opportunistic human infections and diarrhea. This study was isolates from the Masinga Dam during the dry season.

The bacterial isolates were highly sensitive to ciprofloxacin (100%) and gentamycin (98.9%), which aligns with the findings of (Jawahar 2011), whose findings were similar to human bacterial pathogens highly sensitive to ciprofloxacin (91%), chloramphenicol (88%) and gentamycin (85%). The relatively high resistance to ampicillin of 61.5% to most of the isolates is in partial follows with the findings by (Barat et al. 2002), who found the resistance of gram-negative bacteria isolates from fish to ampicillin prevalence of 93.4%, also Newaj-fyzul et al. (2006) findings of predominance resistance to ampicillin of 90.2%. That could be due to the limited use of antibiotics in aquaculture in Kenya. The finding of 31.8% of isolates resistant to tetracycline is comparable with 47% reported by Castro-Escarpulli et al. (2003) for isolates recovered from Tilapia (*O. niloticus*) in Mexico intended for human consumption.

Cow dung manure serves as a potential carrier of pathogenic bacteria as a result of contact with the manure, which is capable of transmitting zoonotic diseases to humans; when this untreated manure is used to fertilize fish ponds, it may serve as a potential source of food-borne infections for the fish consumers and lead to an increase in bacterial infections in the fish. However, resistance to the anti-microbial agents may be because of the widespread, indiscriminate, and lengthy use of chloramphenicol, tetracycline, and gentamicin in cow infection treatments (Omojowo and Omojasola 2013). Sagana fish ponds are fertilized with cow dung manure, which could explain why bacterial isolates from the Masanga dams showed resistance to more antibiotics. Another study concluded

that integrated fish farming favors antimicrobial-resistant bacteria in the pond environment by Andreas et al. (2002). Another study by Anja et al. (2000) found that high levels of individual and multiple anti-microbial resistances within the collected *Flavobacteria* and *Aeromonads* were demonstrated, which indicated a substantial impact on several groups of bacteria associated with aquaculture environments of fish farming.

In conclusions, (i) Fish from both the Masinga Dam and Sagana Ponds dam harbor bacteria and pathogenic bacteria, which naturally inhabit the animal gut flora. In Sagana, *Salmonella, S. boydii,* and *E. coli* were isolates; in Masinga Dam, the isolates were *P. shigelloides* and *E. coli*. (ii) No significant difference of bacteria flora species isolated in the two seasons or the sites. Some bacteria isolates in both seasons were *E. coli, S. boydii, C. freundii, Salmonella spp,* and others. The study shows that bacteria species found in the gut and on fish skin are similar to the bacteria found in cultured fish environments. (iii) The study showed that there was relatively high antibiotic resistance in the isolates to ampicillin of 61.5% and sensitivity to ciprofloxacin (100%) and gentamycin (98.9%). (iv) There was various antibacterial response of isolates from Sagana Ponds but not significantly different in the Masinga Dam.

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