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# Occurrence of potential pathogenic and zoonotic bacteria in farmed fish in Machakos and Nyandarua Counties, Kenya

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**Abstract**
Bacteria can cause diseases in cultured fish. Some are zoonotic hence of public health significance. This study determined occurrence of potential fish pathogens and zoonotic bacteria in farmed fish in Machakos and Nyandarua counties. A cross-sectional study was done. Gills, intestines, skin swabs and kidney swabs from 75 fish and 15 source pond water samples were processed for bacterial culture and identification. These included 40 fish and 8 water samples from Machakos and 35 fish and 7 water samples from Nyandarua. Using conventional methods and Matrix assisted laser desorption/ionization-time of flight mass spectrometry, bacteria isolates were identified. A total of 322 bacterial isolates were identified, 182 from Machakos and 140 from Nyandarua, 299 from fish and 23 from water .They comprised 16 different bacteria genera and 20 species. *Aeromona*s was the most isolated bacterial genus at 32%, *Bacillus* 29%, *Pseudomonas* 21%, *Flavobacterium* 6%, *Micrococcus* 2.8% and *Acinetobacter 2%,*. Fish pathogen genera identified were *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Enterococcus*, *Micrococcus*, *Enterobacter* and *Citrobacter*. Zoonotic bacteria isolated were *Aeromonas hydrophila*, *Aeromonas veronii*, *Escherichia coli*, *Pseudomonas putida*, *Bacillus cereus*, *Citrobacter freundii*, *Hafnia alvei* and *Enterobacter cloaca.* The highest proportion of isolates was from tilapia at 59%, followed by catfish at 35.5% and rainbow trout at 7.5%. Catfish had highest number of zoonotic bacteria genera (6), tilapia (5) and trout (3). Conclusively, fish from both counties harbor zoonotic and pathogenic bacteria which can cause fish and human illnesses. Awareness creation among farmers is paramount. Study data will help in policy development on prevention and control of the respective bacteria.

**Keywords:** Farmed fish, pathogenic and zoonotic bacteria, Nile tilapia, Catfish, Rainbow trout, Nyandarua County, Machakos County

## 1. Introduction
Fisheries and aquaculture sector contribute to food security, employment and foreign exchange. In Kenya it contributes 0.5% of the Gross Domestic Product (FAO, 2015). Fish is one of the most traded food and a valuable source of proteins in the world, Kenya included. Fish farming in Kenya consists of both freshwater and marine aquacultures. The main food fish species cultured in freshwater are Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and rainbow trout (*Oncorhynchus mykiss*). There are also ornamental fish like koi and gold fish. Marine cultured species include milkfish, mullet and crustaceans such as prawns and crabs (KeFS, 2021). Bacterial pathogens are a threat to freshwater, brackish and marine fishes both capture and cultured (Bondad-Reantaso *et al*., 2005) [8] leading to significant losses in the aquaculture industry and fisheries in general (Romalde *et al*., 2019) [28].

The challenge of massive production losses caused by fish diseases is imaginably enormous and horrendous. Besides, some of the bacteria in fish are zoonotic and can cause human illness. Poor water quality, overstocking, lack of biosecurity measures in fish farms, use of contaminated feeds and dirty pond environment are among factors that creates a fertile ground for multiplication of fish bacterial, viral and fungal pathogens resulting in disease outbreaks (Jacobs & Chenia, 2007; Pridgeon & Klesius, 2012) [17]. Members of the genera *Aeromonas*, *Streptococcus*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Enterococcus*, *Enterobacter*, *Citrobacter*, *Vibrio*, *Edwardsiella* and *Mycobacteriium* form a significant

proportion of infectious bacterial organisms in the fish industry (Plumb and Hanson, 2011).

This study aimed at isolation and identification of zoonotic and pathogenic fish bacteria in a bid to advise policy makers on prevention and control of the respective bacteria. This is fundamental and cardinal if profitability and safe consumer aquatic products are to be guaranteed from both aquaculture and wild fisheries.

## 2. Materials and Methods

### 2.1 Study site and design

A cross-sectional study was done. Fish and source pond water samples from various farms in cold (Nyandarua) and warm (Machakos) climates (Figure 1) were collected and processed for bacterial isolation and identification. Machakos lies in latitude $0^0$ 45' South to $0^0$ 31' South and longitude $36^0$ 45' East and $37^0$ 45' East while Nyandarua lies between latitude $0^0$ 32'59.99 North and longitude $36^0$36'59.99 East.

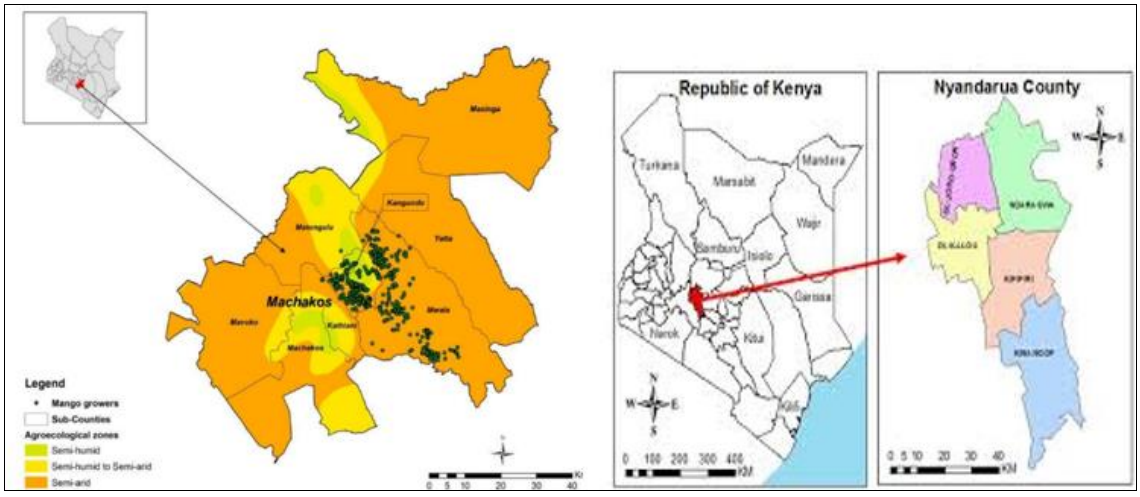


**Fig 1:** Maps of Machakos and Nyandarua counties (map photos courtesy of Semantic scholar, 2015 and 2018)

### 2.2. Sample size determination

Fish and water sample size was determined statistically using the formula: $n = \frac{z^2\ p(1-p)}{d^2}$ (Naing *et al*., 2006) [24] where n was the sample size, z was the z statistic at 95% confidence level, p was the expected prevalence of bacterial pathogens which was assumed to be 50% while d was the precision which was 0.05. This gave a sample size of 384. Due to resource constraints 90 representative samples (40 tilapia, 30 African catfish, 5 rainbow trout and 15 water samples) were collected.

### 2.3 Selection of farms for fish and respective water sample collection

A total of 15 fish farms were selected for fish and pond water sampling according to procedures Yamane (1967). The formula assumes a confidence interval of 95% and a maximum variability of P=0.05. Distribution of the farms was as follows: eight from Machakos and seven from Nyandarua counties.

They were selected from 46 active fish farms identified earlier. In Machakos, two farms were selected from each of four selected sub-counties namely Machakos town, Mavoko, Kangundo and Matungulu while in Nyandarua, one farm from Ndaragwa, two farms from Kinangop, two from Kipipiri and two from Olkalou were selected.

### 2.4. Fish sampling and processing and water sampling

For the eight farms sampled in Machakos County, five live fish were collected from each of the selected farms giving a total of 40 fish (10 Tilapia were collected from Machakos town subcounty, 10 Catfish from Mavoko, 10 tilapia from Kangundo and five catfish, five tilapia from Matungulu subcounty).

For the seven farms sampled from Nyandarua County, five live fish were collected from each of the seven selected farms giving a total of 35 fish (Five rainbow trout, five catfish from Kinangop, ten catfish from Kipipiri, 10 tilapia from Olkalou and five tilapia from Ndaragwa). Overall, 75 live fish (40 tilapia, 30 catfish and five trout) of grow-out to market size were collected using 20 liter capacity buckets and transported to the nearest veterinary laboratories in both Machakos and Nyandarua counties for post mortem examination and sample collection. Four tissue samples (skin, gills, intestines and kidneys) were collected from each fish. Working surfaces were disinfected using 70% ethanol before commencement of postmortem examination. Each fish was first humanely killed; put in a lateral position and using a sterile fenestrated aluminium metal plate, a skin swab was taken. A portion of the gills was also taken. Using a surgical blade and a dissecting kit that had been autoclaved at 121 $^0$C for 15 minutes, a post mortem examination was then conducted as described by Noga (2010). A portion of the intestine and a kidney swab were taken. The fish organ samples including the skin and kidney swabs were put in separate sterile bijou bottles containing Stuart transport media, labeled, kept in cooler boxes packed with ice and transported to the laboratory at the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for bacterial isolation and identification.

Using a sterile universal bottle submerged 20 cm deep into the pond, 20 ml water was collected from each selected farm. The water samples were labelled, packed in a cooler box and transported to the laboratory at the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for bacterial isolation and identification. Overall, 15 water samples were collected.

### 2.5. Preparation of fish and water samples for bacterial isolation

The tissue portions of gills and intestines were pulverized

separately in 4 ml physiological saline using a pestle and a mortar. Half (0.5)ml of the homogenized gills or intestinal tissues were mixed with 4.5 ml of alkaline peptone water (pH 8.4) to obtain a 1:10 dilution each for pre-enrichment and incubated at room temperature for 24 hours. The skin and kidney swabs were also mixed with 4.5 ml alkaline peptone water (pH 8.4) each for pre- enrichment and incubated at room temperature for 24 hours. Half (0.5) ml of water samples was also pre-enriched in 4.5 ml of alkaline peptone water and incubated at room temperature ($24^0$-$26\ ^0$C) for 24 hours.

### 2.6. Culture and Identification

Using a sterile wire loop, the samples were aseptically streaked on separate agar containing blood agar, MacConkey agar and nutrient agars and incubated in inverted position for two days at room temperature. Bacterial colonies were observed for colony morphology, size, color, degree of hemolysis, elevation, shape, lactose fermentation and recorded. Further identification included Gram staining, biochemical tests namely catalase, oxidase, urea, glucose, sucrose, lactose and mannitol fermentation, indole production, methyl red, citrate utilization and production of hydrogen sulphide as described by Cowan and Steel's manual for identification of medical bacteria (1993). Bacterial identification was further confirmed at National Public Health Laboratory using Matrix Assisted Laser Desorption/Ionization –Time of Flight technique.

### 2.7. Statistical analysis

Data was cleaned and entered in an Excel spreadsheet. Both descriptive and analytical statistics were used to analyze the data. Descriptive statistics included frequencies, percentages, tabulations and graphs. Chi-square test of independence using R studio application version 1.4.1717 was used for inferential statistics. All tests were done at a significance level of $P \leq 0.05$

### 2.8. Ethical clearance

Approval to carry-out the study was obtained from the Faculty of Veterinary Medicine Biosafety, Animal use and Ethics Committee, University of Nairobi (Reference number FVM BAUEC/2020/273) and licensed by the National Council for Science, Technology and Innovation in Kenya (Reference number 842968).

## 3. Results

### 3.1. Isolation of bacteria in fish and source pond water

A total of 322 bacterial isolates were identified from 40 Nile tilapia, 30 catfish, five trout and 15 source pond water samples. The bacteria were congregated into 16 genera based on their biochemical characteristics (Table 1).

*Aeromonas* was the most isolated genus at 32%, *Bacillus 29%*, *Pseudomonas* 21%, *Flavobacterium 6%*, *Micrococcus 2.8%*, and *Acinetobacter 2%*; other genera were isolated in lower percentages. In terms of diversity, Machakos had 14 bacteria genera compared to Nyandarua which had 10. Overall, Machakos had 57% while Nyandarua had 43% of the bacteria isolates (Table 1).

**Table 1:** Types, numbers and percentages of bacterial genera isolated from Machakos and Nyandarua counties.

| Bacteria Genus | Machakos (n=182), number of isolates (%) | Nyandarua (n=140), number of isolates (%) | Total counts (n=322), Number of isolates (%) |
|---|---|---|---|
| *Aeromonas* | 47 (26) | 55 (39) | 102(32) |
| *Bacillus* | 61(34) | 32(23) | 93(29) |
| *Pseudomonas* | 28(15) | 40(29) | 68(21) |
| *Flavobacterium* | 17(9) | 2(1) | 19(6) |
| *Micrococcus* | 5(3) | 4(3) | 9(2.8) |
| *Acinetobacter* | 7 (4) | 0 (0) | 7 (2) |
| *Exiguobacterium* | 5(3) | 0(0) | 5(1.6) |
| *Enterococcus* | 3 (2) | 1 (0.7) | 4 (1.2) |
| *Enterobacter* | 2(1) | 1(0.7) | 3 (0.9) |
| *Hafnia* | 3(3) | 0(0) | 3(0.9) |
| *Kurthia* | 0 (0) | 2 (1) | 2 (0.6) |
| *Citrobacter* | 0(0) | 2(1) | 2(0.6) |
| *Rhodococcus* | 1(0.5) | 0(0) | 1(0.3) |
| *Eschherichia* | 1(0.5) | 1 (0.7) | 1 (0.3) |
| *Pseudarthrobacter* | 1 (0.5) | 0 (0) | 1 (0.3) |
| *Lysinibacillus* | 1 (0.5) | 0 (0) | 1 (0.3) |
| TOTAL | 182(57) | 140(43) | 322 |

n is number of isolate

n % is number of isolates expressed as a percentage.

Thirteen bacterial genera were isolated from the urban sub-counties of Machakos compared to only 6 genera that were isolated from the peri-urban sub-counties. In total, 51.6% of the bacterial isolates in Machakos were from urban sub-counties compared to 48.4% that were from peri-urban sub-counties. *Citrobacter* and *Kurthia* were not isolated from both urban and peri-urban subcounties (Table 3).

**Table 2:** Bacterial isolates in urban and peri-urban subcounties of Machakos County

| Bacteria Genus | Urban sub-counties (N=94) Number of isolates (%) | Peri-urban (N=88) Number of isolates (%) |
|---|---|---|
| *Aeromonas* | 34(36.1) | 13(14.7) |
| *Pseudomonas* | 4 (4.3) | 24(27.3) |
| *Flavobacterium* | 17(18.1) | 0(0) |
| *Bacillus* | 14(14.9) | 47(53.4) |
| *Enterobacter* | 1(1.1) | 1(1.1) |
| *Acinetobacter* | 5(5.3) | 2(2.3) |
| *Micrococcus* | 5(5.3) | 0(0) |
| *Enterococcus* | 3(3.2) | 0(0) |
| *Rhodococcus* | 1(1.1) | 0(0) |
| *Escherichia* | 1(1.1) | 0(0) |
| *Hafnia* | 3(3.2) | 0(0) |
| *Exiguobacterium* | 5(5.3) | 0(0) |
| *Pseudarthrobacter* | 1(1.1) | 0(0) |
| *Lysinibacillus* | 0(0) | 1(1.1) |
| TOTALS | 94(51.6) | 88(48.4) |

.n = number of isolates
n% = percentage number of isolates

The warm region of Nyandarua had more bacterial isolates at 38.6%, followed by the moderate temperature areas at 35.7% and lastly cold regions at 25.7%. *Aeromonas* was the main bacteria genus in the cold and moderate temperature regions at 55.6% and 50% respectively. *Pseudomona*s was the most isolated bacterial genus in the warm region of Nyandarua at 59.3%. *Acinetobacter*, *Rhodococcus*, *Hafnia*, *Exiguobacterium*, *Pseudarthrobacter* and *Lysinibacillus* species were not isolated from all the three regions of Nyandarua County (Table 3).

**Table 3:** Bacterial isolates in cold, moderate and warm counties of Nyandarua County

| Bacteria Genus | Cold region (N=36) Number of isolates (%) | Moderate temperature region (N=50) Number of isolates (%) | Warm region(N=54) Number of isolates (%) |
|---|---|---|---|
| *Aeromonas* | 20 (55.6) | 25 (50) | 10 (18.5) |
| *Pseudomonas* | 5 (13.9) | 3 (6) | 32 (59.3) |
| *Flavobacterium* | 0 (0) | 2 (4) | 0 (0) |
| *Bacillus* | 5 (13.9) | 17 (34) | 10 (18.5) |
| *Enterobacter* | 1 (2.8) | 0 (0) | 0 (0) |
| *Micrococcus* | 3 (83.3) | 0 (0) | 1 (1.9) |
| *Enterococcus* | 1 (2.8) | 0 (0) | 0 (0) |
| *Escherichia* | 1 (2.8) | 0 (0) | 0 (0) |
| *Citrobacter* | 0 (0) | 2 (4) | 0 (0) |
| *Hafnia* | 0(0) | 0(0) | 0(0) |
| *Kurthia* | 0 (0) | 1 (2) | 1 (1.9) |
| Totals | 36(25.7) | 50 (35.7) | 54(38,6) |

n = number of isolates
n% is the number of isolates calculated as a percentage

Most of the bacterial isolates were in the gills at 26.1%, followed by intestines 24.2%, skin 22.4%, kidneys 20.2% and water at 7.1%. *Aeromona*s was the most isolated bacteria genus from the intestines and water at 39.7% and 43.5% respectively. *Bacillus* was most isolated bacteria genus from the skin and kidneys at 30% and 38.5% respectively while *Pseudomonas* was the most isolated genus from gills at 27.4% (Table 4). Tilapia had 65% of skin isolates, Catfish 26.3% and trout 8.3%.For the gill isolates, Tilapia had 52%, Catfish 40% and Trout 7.4%. In the intestines, Tilapia had 64% isolates, Catfish 28.2% and Trout 7.7% while for the kidney isolates Tilapia had 70%, Catfish 20% and Trout 9.2%. Machakos had 57% of the skin isolates, 55% gill isolates, 68% intestinal isolates, 51% kidney isolates and 61% of water isolates while Nyandarua had 43% skin isolates,45% gill isolates,32% intestinal isolates,49% kidney isolates and 39% of the water isolates

**Table 4:** Type, numbers and percentages of bacterial isolates from fish organs and source water.

| Bacteria isolate | Skin (n=72), n(%) | Gills (n=84), n(%) | Intestine (n=78) n(%) | Kidneys (n=65) n(%) | Water (n=23) n(%) |
|---|---|---|---|---|---|
| *Aeromonas spp* | 20(6.2) | 22(6.8) | 31(9.6) | 19(5.9) | 10(3.1) |
| *Pseudomonas spp* | 19(5.9) | 23(7.1) | 14(4.3) | 10(3.1) | 2 (0.6) |
| *Flavobacterium* | 4(1.2) | 3(0.9) | 7(2.2) | 2(0.6) | 3(0.9) |
| *Bacillus spp* | 21(6.5) | 22(6.8) | 18(5.6) | 25(7.8) | 7(2.2) |
| *Enterobacter spp* | 0(0) | 2(0.6) | 0(0) | 0 (0) | 1 (0.3) |
| *Acinetobacter spp* | 1(0.3) | 1(0.3) | 3(0.9) | 2(0.6) | 0(0) |
| *Micrococcus spp* | 1(0.3) | 2(0.6) | 1(0.3) | 5(1.6) | 0(0) |
| *Rhodococcus spp* | 0(0) | 1(0.3) | 0(0) | 0(0) | 0(0) |
| *Escherichia spp* | 0(0) | 2(0.6) | 0(0) | 0(0) | 0(0) |

| | | | | | |
|---|---|---|---|---|---|
| *Citrobacter spp* | 1(0.3) | 1(0.3) | 0(0) | 0(0) | 0(0) |
| *Hafnia spp* | 1(0.3) | 0(0) | 1(0.3) | 1(0.3) | 0(0) |
| *Kurthia spp* | 1(0.3) | 1(0.3) | 0(0) | 0(0) | 0(0) |
| *Exiguobacteria* | 1(0.3) | 2(0.6) | 1(0.3) | 1(0.3) | 0(0) |
| *Pseudarthrobacter* | 0(0) | 0(0) | 1(0.3) | 0(0) | 0(0) |
| *Lysinibacillus spp* | 0(0) | 1(0.3) | 0(0) | 0(0) | 0(0) |
| *Enterococcus spp* | 2(0.6) | 1(0,3) | 1(0,3) | 0(0) | 0(0) |
| Total | 72 (22.4) | 84(26.1) | 78(24.2) | 65(20.2) | 23(7.2) |

n is the number of isolates; n % is the number of isolates expressed as a percentage

### 3.2. Occurrence of Fish bacterial pathogens and zoonotic bacteria

Matrix assisted laser desorption/ionization-time of flight technique identified eight species that were zoonotic namely *Aeromonas hydrophila, Aeromonas veronii, Bacillus cereus, Escherichia coli, Pseudomonas putida, Hafnia alvei, Enterobacter cloaca* and *Citrobacter freundii*. Out of the eight species, three were only found in Machakos namely *Pseudomonas putida, Enterobacter cloaca* and *Hafnia alvei*; two were found only in Nyandarua namely *Aeromonas veronii* and *Citrobacter freundii* while three species were found in both counties namely *Aeromonas hydrophila, Bacillus cereus* and *Escherichia coli*. Of the eight zoonotic species identified, four were found in both Tilapia and Catfish namely *Aeromonas hydrophila, Escherichia coli, Bacillus cereus* and *Hafnia alvei*; two were found in Tilapia namely *Pseudomonas putida* and *Aeromonas veronii*; *Enterobacter cloaca* was found in Tilapia, Catfish, Trout and water while *Citrobacter freundii* was found only in catfish. A total of nine bacterial genera whose some of its members are potential fish pathogens were also identified. These included *Aeromonas, Pseudomonas, Citrobacter, Enterococcus, Micrococcus, Acinetobacter, Enterobacter, Hafnia* and *Flavobacterium.* Out of the nine genera, six were isolated from both counties namely *Aeromonas, Pseudomonas, Flavobacteium, Enterobacter, Micrococcus* and *Enterococcus*; two were isolated from Nyandarua only namely *Citrobacte*r and *Hafnia* while *Acinetobacter* was isolated from Machakos only. Of the nine fish pathogenic genera, four were isolated from Tilapia, Catfish and Trout namely *Aeromonas, Pseudomonas, Enterobacter* and *Micrococcus*, four were isolated from Tilapia and catfish namely *Flavobacterium, Acinetobacter, Enterococcus* and *Hafnia* while *Citrobacter* was isolated from Catfish only. Bacterial genera that was isolated from water included *Aeromonas, Pseudomonas, Flavobacterium* and *Enterobacter*. Isolates in water served as a potential source of contamination to the fish in the pond. *Aeromonas, Pseudomonas* and *Flavobacterium* were present in water and in kidneys, intestines, gills and skin thus making the pond water a source of infection to the fish. *Enterobacter* was present in water and gills only making it either an infection or a contaminant from the aquatic environment, Overall, there were more bacterial isolates from Machakos compared to Nyandarua thus making Nyandarua have less threats of both fish pathogens and zoonosis. The warm climate of Machakos favored growth of mesophilic bacteria like *Escherichia coli, Aeromonas species, Pseudomonas species* and *Enterococcus species.*

## 4. Discussion

Bacteria are potential fish pathogens that cause tremendous losses in fish farming through diseases. It is imperative to understand the microbiological status of cultured fish to develop preventive and control strategies for successful aquaculture to be realized. Sixteen bacteria genera were identified from this study. This agreed with the findings of Predgeon & Klesius (2012) [17] who reported that there are more than 13 bacterial genera which can cause bacterial diseases in the aquaculture industry globally; In addition, the study findings are in agreement with observations from Wanja *et al*., (2020) [32] who reported 17 bacterial genera from cultured fish in Kirinyaga County, Kenya. Nine of the bacterial genera reported in this study were the same as those reported by Wanja *et al*., (2020) [32]. However, there were seven genera reported in this study that had not been reported earlier. These were *Hafnia, Kurthia, Exiguobacterium, Pseudarthrobacter, Lysinibacillus, Rhodococcus and Enterococcus.* Machakos had more bacterial isolates in terms of population and diversity than Nyandarua this is because it is warmer in Machakos compared to Nyandarua. Similarly, the warmer areas of Nyandarua County had more bacterial isolates than the cold areas. Warmer temperature favor bacterial multiplication (Dixon *et al*., 2012). In Machakos County, the urban subcounties of Machakos town and Mavoko had relatively more bacterial isolates (54%) compared to the peri-urban counties of Kangundo and Matungulu (46%). This may have been due to poor water quality of the fish ponds in urban subcounties caused by environmental contamination and pollution. Water pollution increases susceptibility of farmed fish to bacterial infection (Sarmento *et al*., 2004)

Most of the bacteria were isolated from the gills (26.1%) which was in line with the findings of Wanja *et al*., (2020) [32]. It was followed by the intestines at 24.2%, skin 22.4%, kidneys 20.2% and water 7.1%. Having the least number of bacteria in the kidneys was also observed by Wanja *et al*., (2020) [32]. This implies that about 20% of the bacteria were septicaemic. The four leading bacterial genera identified thus *Aeromonas, Pseudomonas, Bacillus* and *Flavobacterium* were also found in the source pond water. This confirms a proportionate relationship between bacterial composition of pond water and that found in the fish (Apun *et al*., 1999) [7]. This may be due lack of pond disinfection before stocking or restocking, never changing pond water, use of untreated or polluted water sources for the pond. Bacteria isolates were not specific to fish type as the most isolated genera were found in all the three fish species namely Nile tilapia (54%), African catfish (32%) and rainbow trout (6.9%); water had 7.1% of the isolates in this study.

In this study, *Aeromonas hydrophila* and *Aeromonas veronii* were isolated from fish. Both species are potential fish pathogens (Zhu *et al*., 2016; Chondrarantha *et al*., 2018). Members of the genus *Aeromonas* including *Aeromonas hydrophila, Aeromonas veronii, Aeromonas caviae, Aeromonas sobria* and *Aeromonas schuberti* cause disease in pond raised fish (Deen *at al*., 2014). Other bacteria isolated from the fish were *Pseudomonas putida, Enterococococcus feacalis, Micrococcus luteus, Citrobacter freundii, Citroacter braaki, Enterobacter cloaca, and Flavobacterium columnaris,* These have also been documented as potential fish pathogens (Eissa *et al*., 2010; Novais *et al*., 2018; Austin and Stobie, 1992; Zurfluh *et al*., 2017; Li *et al*., 2017; Sekar *et al*., 2008;

Suomalainen *et al.*, 2005; Faisal *et al*., 2017). These fish pathogens can cause massive losses in aquaculture.Some of the bacteria isolated and identified are zoonotic hence are of public health significance. These included *Aeromonas hydrophila and Aeromonas veronii*, (Janda and Abbort, 2010) *Citrobacter freundii* (Liu *et al*., 2017a), *Enterobacter cloaca* (Wang *et al*. 2012), *Hafnia alvei* (Hastein *et al*., 2006), *Escherichia coli* (Beuchat, 1996), *Bacillus cereus* (Schoeni & Wong, 2005*) and Pseudomonas putida* (Evans *et al.*, 2006) [17]. *Pseudomonas mendocina* is neither a fish pathogen nor zoonotic while *Pseudomonas putida* is both a fish pathogen and zoonotic (Evans *et al*., 2006) [17]. Occurrence of *Citrobacter braaki, Pseudomonas putida, Aeromonas veronii* and *Hafnia alvei* in fish in Kenya was reported for the first time in this study.

## 5. Conclusions and Recommendations

Pond raised fish in Nyandarua and Machakos counties have potential pathogenic bacteria that can cause disease in fish. Some of the bacteria have zoonotic potential and can infect humans through consumption of raw or undercooked fish hence are of public health significance. Warmer areas and urban farms investigated had more potential fish pathogens and zoonotic bacterial organisms than cooler and peri-urban areas. Robust awareness creation on good aquaculture management practices and capacity building among fish farmers through extension services is necessary. Appropriate policy and legislation is required to ensure fish welfare and wellbeing, and safe fish and fish by-products for human consumption.

## 6. Conflicts of interest

The authors declare that there are no conflicts of interest.

## 7. Acknowledgements

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