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Bacterial pathogens isolated from farmed fish and source pond water in Kirinyaga County, Kenya

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Abstract
Bacterial infections cause low to high mortality in fish, affecting the productivity of aquaculture. This study aimed at determining the occurrence of bacterial pathogens in farmed tilapia, catfish, goldfish and koi carp and source pond water in Kirinyaga County. A total of 181 healthy-appearing fish and 27 water samples from randomly selected fish farms in the county were processed. Bacteriological isolation was done on aseptically collected skin and kidney swabs; gills and a portion of intestines from each fish and water samples. Isolated bacteria were identified by colony morphology, Gram stain and biochemical characteristics, and some further characterized using API-20E kit. A total of 329 bacterial isolates were recovered from fish organs and 39 from pond water samples. They belonged to 17 genera with 18 different identified bacterial species. The most prevalent species found on the skin, gills, intestines, kidney, and water samples belonged to five genera: *Proteus* spp. (14.9%), *Aeromonas hydrophila* (8.2%), *Aeromonas caviae* (6.3%), *Plesiomonas* (5.2%), *Flavobacterium* spp. (5.2%), *Aeromonas sobria* (4.3%) and *Micrococcus* spp. (4.3%). Some isolates (11%, n=42) could not be identified. Bacterial species recovered from fish samples were also found in the water samples except: *Streptococcus* spp., *Pseudomonas luteola, Serratia plymuthica* and *Klebsiella oxytoca. Raoultella terrigena* was recovered from water samples only.
The study has shown that farmed fish and aquatic environments harbor potentially pathogenic and zoonotic bacteria which may cause significant fish diseases and public health risks. Therefore, there is need to implement stringent management and biosecurity programs.

Keywords: Bacterial infections, aquaculture, fish diseases, public health, biosecurity

1. Introduction
Kenya has fast growing fish species (*Oreochromis niloticus*, *Clarias gariepinus* and *Oncorhynchus mykiss*) aquaculture industry. The country is among the major aquaculture producers of sub-saharan Africa, with an industry dominated by tilapines [1]. The capture and wild fisheries contributes 0.8% of the Gross Domestic Product (GDP), providing direct employment opportunities to over 500,000 people and supporting over two million people indirectly [1]. However, fish supply in Africa has been declining for a number of reasons while the demand has increased due to rapidly growing human population. In an effort to reverse these trends, aquaculture projects have been massively promoted across the continent, Kenya included. This is characterized by a record growth extensive small scale to intensive large scale fish farms over the last decade. One significant setback for rapid intensification in aquaculture is risk of diseases, caused by parasites [2-4], viruses [5], fungi [6] and bacteria.
Although aquaculture may not be exclusively implicated for the rising disease concern in fisheries, it does provide key insights into: how the diseases may be spread, maintained and whether it is significant enough to elicit action. Of the diseases, bacteriosis remains the most damaging to fish production globally due to economic significance of diseases they cause [7]. There has been a steady increase in the number of species of bacteria implicated in causing fish diseases. An estimated 125 different bacterial species belonging to 34 different bacterial families has been reported to cause various fish diseases globally [8], including: *Aeromonas* spp., *Vibrio* spp., *Pseudomonas* spp., *Yersinia* spp., *Flavobacterium* spp., *Renibacterium* spp., *Mycobacterium* spp., *Edwardsiella* spp., *Citrobacter* spp. and *Streptococcus* spp. [9]. However, there is growing indication that the pathogenic species spectrum as well as the geographic and host range is widening among fish pathogens [10], leading to the emergence of new pathogens.

Most documented cases of fish diseases in Kenya have narrowed their focus to parasitic infections in capture and wild fish [11], and may thereby miss out on potentially important disease-causing bacterial microbes. Concomitant to this; it is essential to monitor the health of fish stock so as to produce fish that is safe for human consumption There is scarce information available on the occurrence of bacterial pathogens affecting the aquaculture sector in Kenya. While most published studies have focused on isolation of single bacteria species; this study aims to isolate and identify common bacterial pathogens in farmed food and ornamental fish and pond water in Kirinyaga County, Kenya. Studies on aquatic bacterial flora would provide information relevant in developing more stringent biosecurity and sanitary measures.

2. Materials and Methods

2.1 Ethical approval

This research work was approved by the Faculty of Veterinary Medicine Biosafety, Animal use and Ethics Committee for Experimentations on live non-human vertebrates, University of Nairobi, Kenya.

2.2 Study area and design

A cross sectional study was carried out where fish and water samples were collected from five sub-counties of Kirinyaga County, between December 2017 and April 2018. The sub-counties involved were Mwea East, Mwea West, Ndia, Gichugu, Kirinyaga West and Kirinyaga Central (Figure 1). Bacteria were isolated from the sampled fish and source pond water and identified by colony morphology, Gram stain and biochemical characteristics, while some were further characterized using Analytic Profile Index (API) 20E microorganism identification kit.

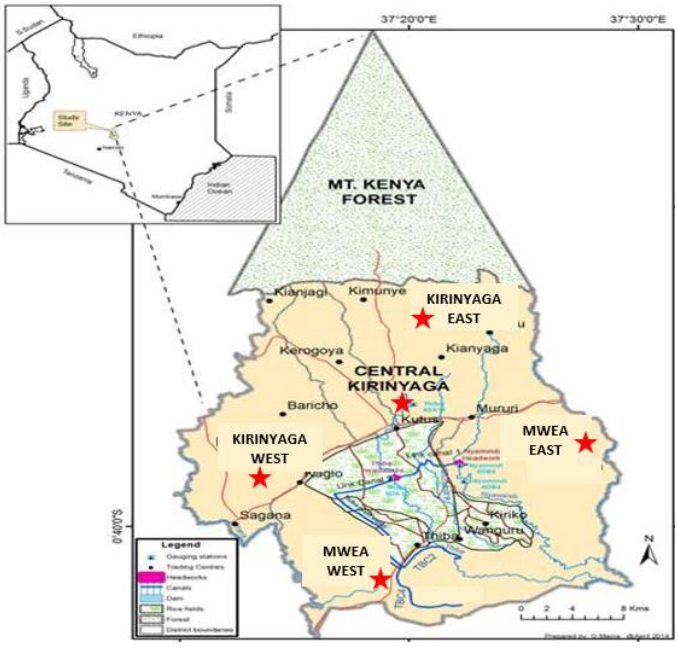

Fig 1: Kirinyaga County map showing the five sub counties (stars) visited during the study. Map modified from Serede *et al.* [12].

2.3. Sampling procedure and sample size

Simple random sampling was used to select active and available farms in the study area. The sample size was calculated using the formula given by Naing *et al.* [13]; $n = \frac{Z^2 P(1-P)}{d^2}$, where n is the sample size, Z is the Z statistic for a level of confidence (1.96 for 95%), P is expected prevalence (assumed pathogen prevalence level of 50%) and d is the precision, which is equal to 5% (0.05). This gave a sample size of 384. However, only 208 samples comprising: 88 tilapia, 53 catfish, 30 goldfish and 10 koi carp and 27 source pond water samples were collected, owing to limitations in resources and time. Fifteen grow-out farms and five breeder farms were sampled based on availability and consent of farmers and number of active fish ponds. To obtain a proportional sample, 5-10 fish were purchased per pond from selected farmers. Live fish were transported to Kerugoya County Veterinary Department Laboratory for necropsy in sterile 18 litre buckets.

Surface pond water samples were collected from the same ponds where fish sampling was done. The water samples were collected aseptically using a sterile 50ml screw capped universal glass bottles, submerged 15cm to 20 cm below the water surface at every sampling. Bottles with water samples were labeled accordingly. Water samples were placed in a cool box packed with ice, then transported to laboratory. The samples were later transferred to a refrigerator before commencing bacteriological examination.

2.4 Collection of fish organ samples

Sampled fish were humanely killed and post mortem examination was done following standard procedures by Noga [14] and Roberts [15]. A square inch of the fish skin surface was swabbed with sterile cotton swab, using a sterile aluminium metal plate as a guide (Figure 2). Prior to opening the body cavity, the ventro-lateral surface of the fish was decontaminated by swabbing with 70% alcohol. The fish were then dissected; and a portion of gills and intestines and kidney were taken aseptically from individual fish for bacteriological analysis.

Fig 2: Tilapia, swabbing a square inch skin surface, guided by a sterile fenestrated aluminium metal plate (red arrow)

2.5 Sample processing and isolation of fish bacteria

All bacteriological analysis was done at the bacteriology laboratory, department of Veterinary Pathology Microbiology and Pathology, University of Nairobi. The samples (skin, gills, intestines, kidney and water) were first brought out of the refrigerator, left outside to warm up to room temperature (24-26ºC). Samples of intestine and gills of each fish were aseptically pulverized separately in sterile physiological saline, using a sterile pestle and mortar. The homogenate and swabs were inoculated in freshly prepared alkaline peptone water (APW; pH 8.4). Source pond water sample was also pre-enriched in APW. Inoculated broth was incubated aerobically at room temperature (24ºC-26ºC) for 2 days.

Growth (taken as turbidity) was checked on APW broth previously inoculated. Using a flame sterilized nichrome wire loop, an inoculum was sub-cultured by streaking separately and aseptically onto plates containing nutrient agar, 10% sheep blood agar and MacConkey agar. The plates were

incubated aerobically at room temperature (24 ºC-26 ºC), in an inverted position. After 24 to 48 hours of incubation, the plates were examined and the colony morphology on the plates recorded. Distinct colonies were sub-cultured to obtain pure colonies. Single colonies from respective isolates were then sub-cultured onto nutrient agar slants. The slants were stored at 4ºC for later use. Suspect *Salmonella* and *Streptococcus* strains were sub-cultured on Salmonella Shigella agar (SSA) and Sodium azide crystal violet blood agar (SACVBA), respectively.

2.6 Identification of isolated bacteria

The isolates were identified using colony morphology and Gram staining characteristics and conventional biochemical tests following Austin and Austin [9] and Bergey's Manual of Determinative Bacteriology [16] and by use of commercially available API 20E kits. Distinct colonies on culture media were examined for phenotypic characteristics (colonial morphology, pigmentation, shape, size, haemolysis and growth on MacConkey agar). Colonies representative of each type of bacterium were stained by Gram's method and examined microscopically. Primary identification of the bacterial isolates was by Gram's reaction, catalase and oxidase production. This was followed by secondary identification that entailed biochemical characteristics using conventional tests that included indole, methyl red test, Simmon's Citrate utilization, degradation of urea, sulphur indole motility (SIM) test and acid production from glucose, sucrose, mannitol and arabinose. Further characterisation of Gram negative bacteria were carried out using the API 20E microorganism identification kit; as described by the manufacturer (BioMérieux Marcy-l'Étoile, France).

2.7 Data management and analysis

Data obtained was entered in Ms. Excel sheets, cleaned and exported to IBM SPSS version 22.0 for analysis using analysis of variance (ANOVA). All the tests were carried out at a significance level of $p<0.05$ to indicate significant differences.

3. Results

3.1 Prevalence of bacterial pathogens in skin, gills, intestines, kidney and water samples

Three hundred and twenty-nine bacterial isolates were recovered and characterized from 181 apparently healthy looking fish and 27 source pond water samples. The bacterial isolates belonged to 17 genera with at least 18 different bacterial species. The occurrence of different bacterial genera and species in the gills, skin, intestines, Kidney and water samples is summarized in Table 1. These bacterial organisms were isolated from gills (23.4%), skin (23.1%), intestine (22.6%), kidney (20.3%) and water samples (10.6%). Most of the bacterial species were Gram-negative bacilli including: *Proteus* spp. (14.9%), *Aeromonas hydrophila* (8.2%), *Aeromonas caviae* (6.3%), *Plesiomonas* (5.2%), *Flavobacterium* spp. (5.2%), *Aeromonas sobria* (4.3%); while a few were Gram positive organisms including genera: *Micrococcus* (4.3%), *Bacillus* (4.1%) and *Streptococcus* (1.9%).

Table 1: Frequency of bacterial pathogens in skin, gills, intestines, Kidney and water samples

	Skin n (%)	Gills n (%)	Intestines n (%)	Kidney n (%)	Water n (%)	Total n (%)
Bacillus spp.∗	3 (0.8)	5 (1.4)	3 (0.8)	3 (0.8)	1 (0.3)	15 (4.1)
Micrococcus spp.∗	3 (0.8)	2 (0.5)	2 (0.5)	7 (1.9)	2 (0.5)	16 (4.3)
Streptococcus spp.	1 (0.3)	3 (0.8)	1 (0.3)	2 (0.5)	–	7 (1.9)
Aer. sobria∗	3 (0.8)	5 (1.4)	3 (0.8)	4 (1.1)	1 (0.3)	16 (4.3)
Aer. hydrophila∗	2 (0.5)	11 (3.0)	8 (2.2)	3 (0.8)	6 (1.6)	30 (8.2)
Aer. caviae∗	4 (1.1)	7 (1.9)	6 (1.6)	5 (1.4)	1 (0.3)	23 (6.3)
Ps. Luteola	2 (0.5)	2 (0.5)	–	–	–	4 (1.1)
Ps. Aeruginosa	5 (1.4)	4 (1.1)	2 (0.5)	–	1 (0.3)	12 (3.3)
Ps. fluorescens∗	1 (0.3)	2 (0.3)	2 (0.5)	4 (1.1)	4 (1.1)	13 (3.5)
Plesiomonas spp.∗	3 (0.8)	8 (2.2)	6 (1.6)	1 (0.3)	1 (0.3)	19 (5.2)
Flavobacterium spp.∗	10 (2.7)	3 (0.8)	1 (0.3)	4 (1.1)	1 (0.3)	19 (5.2)
Proteus spp.∗	15 (4.1)	13 (3.5)	16 (4.3)	9 (2.4)	2 (0.5)	55 (14.9)
Citrobacter freundii∗	5 (1.4)	1 (0.3)	4 (1.1)	3 (0.8)	1 (0.3)	14 (3.8)
Escherichia coli	–	1 (0.3)	2 (0.5)	1 (0.3)	2 (0.5)	6 (1.6)
Salmonella Enteritidis∗	1 (0.3)	1 (0.3)	3 (0.8)	1 (0.3)	1 (0.3)	7 (1.9)
Kleb. Oxytoca	1 (0.3)	2 (0.5)	–	–	–	3 (0.8)
Kleb. pneumoniae∗	3 (0.8)	1 (0.3)	6 (1.6)	4 (1.1)	1 (0.3)	15 (4.1)
Raoultella terrigena	–	–	–	–	1 (0.3)	1 (0.3)
Ser. Plymuthica	1 (0.3)	–	–	–	–	1 (0.3)
Ser. ficaria∗	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	5 (1.4)
Ser. Marcescens	1 (0.3)	3 (0.8)	–	2 (0.5)	1 (0.3)	7 (1.9)
Ser. Liquefaciens	–	–	–B	1 (0.3)	1 (0.3)	2 (0.5)
Enter. Sakazakii	2 (0.5)	–	2 (0.5)	4 (1.1)	2 (0.5)	10 (2.7)
Enter. Cloacae	2 (0.5)	–	1 (0.3)	5 (1.4)	3 (0.8)	11 (3.0)
Pantoea spp.∗	1 (0.3)	1 (0.3)	1 (0.3)	1 (0.3)	2 (0.5)	6 (1.6)
Acinetobacter spp.∗	2 (0.5)	1 (0.3)	1 (0.3)	3 (0.8)	2 (0.5)	9 (2.4)
Unidentified	13 (3.5)	9 (2.4)	12 (3.2)	7 (1.9)	1 (0.3)	42 (11.4)
Total n (%)	85 (23.1)	86 (23.4)	83 (22.6)	75 (20.3)	39 (10.6)	368

Aer: Aeromonas; Ps: Pseudomonas; Kleb: Klebsiella; Ser: Serratia; Enter: Enterobacter

∗Bacteria isolated from both fish tissue and water samples

The phenotypic and biochemical characteristics of bacterial isolates from farmed fish and source pond water are summarized in Table 2. Majority of the isolates were Gram negative bacilli and a few were Gram positive.

Table 2: Biochemical and phenotypic characteristics of bacterial isolates from farmed fish and source pond water

	Gram stain	Catalase	Oxidase	Indole	MR	Citrate	H_2S	Motility	Urease	Glucose	Sucrose	Mannitol	Arabinose	Haemolysis	Growth on MacConkey	Other features
Bacillus spp.	+	+	-	-	-	-	-	-	-	-	-	-	-	H	NLF	Giant
Micrococcus spp.	+	+	-	-	-	-	-	-	-	-	-	-	-	NH	NLF	Pale yellow
Streptococcus spp.	+	-	-	+	+	-	-	-	+	+	+	-	-	H	LF	Pinpoint purple colonies on SACVBA
A. sobria	-	+	+	+	-	+	-	+	-	+	+	+	-	H	LF	
A. hydrophila	-	+	+	+	-	+	-	+	-	+	+	+	+	H	LF	
Ps. aeruginosa	-	+	+	-	-	+	-	+	+	-	-	-	-	H	NLF	Green
Ps. Fluorescens	-	-	+	-	-	+	-	+	+	-	+	-	-	NH	NLF	
Plesiomonas	-	+	-	+	-	-	-	+	-	+	-	-	-	H	LF	
Flavobacterium spp.	-	+	-	-	-	-	-	-	-	-	-	-	-	NH	NLF	Yellow pigmented colonies
Proteus spp.	-	+	-	-	+	-	+	+	+	+	-	-	-	NH	NLF	Swarming
C. freundii	-	+	-	-	+	-	+	+	-	+	+	+	+	NH	LF	
Escherichia coli	-	+	-	+	+	-	+	+	-	+	-	+	+	NH	LF	
Salmonella Enteritidis	-	+	-	-	+	+	+	+	-	+	-	+	+	NH	NLF	Colonies with black centers on SSA
Klebsiella spp.	-	+	-	-	-	+	-	-	+	+	+	+	+	NH	LF	Mucoid
Raoultella terrigena	-	+	-	-	+	-	+	-	+	+	+	+	+	NH	LF	Irregular mucoid
Serratia spp.	-	+	-	-	-	+	-	+	-	+	+	+	-	H	NLF	*S. marcescens* has pink-red
Enterobacter spp.	-	+	-	-	-	+	-	+	-	+	+	+	+	NH	LF	*E. sakazakii* has yellow colonies; Mucoid
Pantoea spp.	-	+	-	-	+	+	-	+	-	+	+	+	+	NH	LF	Yellow
Acinetobacter spp.	-	+	-	-	-	-	-	-	-	-	-	-	-	NH	NLF	

NH: Non-hemolytic; H: Hemolytic; NLF: Non-lactose fermenter; LF: Lactose fermenter; (+): Positive; (-): Negative; SACVBA: Sodium azide crystal violet blood agar; SSA: Salmonella Shigella agar

Figure 3 shows the colony morphology of some isolates in different culture media. *Bacillus* spp. produced dry, giant (amoebic like) and hemolytic colonies on blood agar (Fig. 3A). *Aeromonas hydrophila* colonies are off white, circular β-hemolytic on blood agar plate (Fig. 3B). *Proteus* spp. has a characteristic swarming following spot inoculation in the center of blood agar plate (Fig. 3C). *Flavobacterium* spp. colonies are non-hemolytic and yellow pigmented on blood agar (Fig. 3D). *Serratia marcescens* colonies produce pink-red pigment (Prodigiosin) on nutrient agar (Fig. 3E). *Micrococcus* spp. forms yellow pigmented colonies on nutrient agar (Fig. 3F). *Klebsiella* spp. forms mucoid lactose fermenter (pink) colonies on MacConkey agar plate (Fig. 3G). *Salmonella* Enteritidis produces colonies with black centers on salmonella shigella agar (Fig. 3H). *Streptococcus* spp. forms pinpoint purple colonies on sodium azide crystal violet blood agar (Fig. 3I).

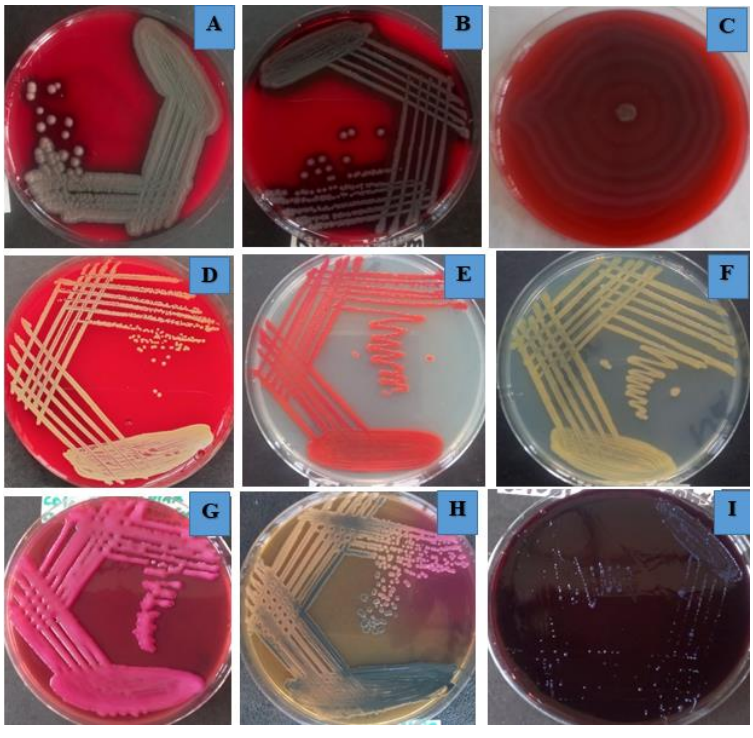

Fig 3: Plates showing colony morphology of some isolates in different culture media.

Isolates identified as aeromonads, pseudomonads and members of the family Enterobacteriaceae were characterized further to species or genus level using API 20E microorganism identification kit.

3.2 Occurrence of bacterial pathogens in fish organs sampled

Bacteriological examination of the various organs revealed that 9 bacterial isolates including: *Micrococcus* spp., *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas caviae*, *Plesiomonas* spp., *Klebsiella pneumoniae*, *Enterobacter sakazakii* and *Acinetobacter* spp., were isolated in the organs of fish (tilapia, catfish, gold fish and koi carp) studied (Table 3), in varying magnitudes.

Table 3: Presence of bacterial isolates in fish organs samples

Bacterial strain	Bacterial growth															
	Nile tilapia				African catfish				Gold fish				Koi carp			
	Sk	Gi	Inst	Kd	Sk	Gi	Inst	Kd	Sk	Gi	Inst	Kd	Sk	Gi	Inst	Kd
Bacillus spp.	+	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-
Micrococcus spp.	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-
Streptococcus spp.	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
Aer. Sobria	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aer. Hydrophila	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aer. Caviae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ps. Luteola	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ps. aeruginosa	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	-
Ps. fluorescens	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Plesiomonas spp.	+	+	+	-	+	-	+	-	-	+	+	+	-	+	+	-
Flavobacterium spp.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Proteus spp.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Citrobacter freundii	+	+	+	-	+	-	+	+	-	-	+	-	-	-	-	+
Escherichia coli	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
Salmonella Enteritidis	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Kleb. oxytoca	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-
Kleb. pneumoniae∗	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raoultella terrigena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ser. plymuthica	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ser. ficaria∗	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Ser. marcescens	+	+	-	+	+	+	-	+	+	+	-	+	-	-	-	-
Ser. liquefaciens	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Enter. sakazakii	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+
Enter. cloacae	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-
Pantoea spp.∗	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Acinetobacter spp.∗	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Sk: Skin; Gi: Gills; Inst: Intestines; Kd: Kidney; (+): Positive; (-): Negative; *Aer*: *Aeromonas*; *Ps*: *Pseudomonas*; *Kleb*: *Klebsiella*; *Ser*: *Serratia*; *Enter*: *Enterobacter;*

∗Bacterial isolates recovered from all the fish species sampled

4. Discussion

Studies on bacterial flora in farmed fish helps in making relevant decisions on respective management and disease control strategies, since some of the organisms are of public health importance. In this study, a total of 17 genera with 18 different identified bacterial species were isolated from fish and respective water samples. The prevalent bacterial flora were Gram-negative bacilli. This agrees with previous studies by Al-Harbi and Naim Uddin [17] who reported 17 genera of bacteria in hybrid tilapia from Saudi Arabia. Nine (53%) genera of bacteria were the same as those previously reported by Al-Harbi and Naim Uddin [17]. However, Karimi, [18] reported 15 genera from tilapia and catfish in Sagana fish farm and Masinga dam in Kenya, with 9 (53%) genera common between this study and Karimi's study. In another study; Wamala *et al*. [19] reported 15 bacteria genera from wild and cultured tilapia and catfish in Uganda. Ten (58%) genera isolated by Wamala *et al*. [19] were common with the ones isolated in this study. Seven of the bacteria identified in the present study are being reported for the first time in fish and aquatic environments from Kenya; they include: *Serratia plymuthica*, *Serratia ficaria*, *Pseudomonas luteola*, *Serratia marcescens*, *Klebsiella oxytoca* and *Raoultella terrigena*. A reason for this finding is that most studies on fish diseases in Kenya have concentrated their focus to parasitic infections in fish, and may thereby miss out on potentially important disease-causing bacterial microbes.

Bacterial species recovered from fish organ samples were also found in the water samples except: *Streptococcus* spp., *Klebsiella oxytoca*, *Pseudomonas luteola* and *Serratia plymuthica*. This confirms that source pond water has an influence on the composition of bacterial flora in fish. These findings are in partial agreement with Apun *et al*. [20] who reported similarity between the microbial content of pond water and fish organs. *Raoultella terrigena* (previously known as *Klebsiella terrigena*) was recovered from water samples only. It is a hardly encountered Gram-negative bacterium and primarily reported as aquatic and soil organism. It has been incriminated to cause post-operative sepsis in human patients [21, 22].

The distribution of the recovered bacterial isolates revealed that the highest number of isolates was found in the gills, skin followed by intestine and then the kidney. With an exception of *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Pseudomonas luteola* and *Serratia plymuthica*, all the other bacterial isolates were recovered from the kidney. This strongly suggests that these bacteria occur or are ubiquitous in the farmed fish aquatic environments. Presence of large

number of bacteria in the kidney can be an indication of infection after overcoming the fish's defense mechanisms or a case of spread via hematogenous route.

The bacterial diversity revealed at least 9 bacterial strains were common in the studied fish (Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), goldfish (*Carassius auratus*) and koi carp (*Cyprinus carpio carpio*) regardless of the fish organ. This finding affirms that ichtyofauna may be affected by similar bacterial community.

Some of the bacterial species identified are of economic importance i.e. known to be pathogenic to fish; causing infectious diseases elsewhere; they include: *Aeromonas hydrophila*, *A. sobria*, *A. caviae*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Citrobacter freundii* [9, 23-28]. Occurrence of these bacterial species in the fish is, therefore, a threat to aquaculture development as may cause low-to-high mortality depending on the environmental culture conditions.

The presence of members of family Enterobacteriaceae, mainly *Salmonella* Enteritidis, *Citrobacter freundii*, *Enterobacter aerogenes*, *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Raoultella terrigena* in fish pose health risk to humans [29, 30]. A study by Ampofo *et al.* [31] have shown that entero-pathogens are common in organic waste-fertilized ponds. The high prevalence in *Proteus* spp. isolated may be associated with use of poultry litter [32] to fertilize fish ponds. Pretreatment of manure by exposing to sunlight is therefore recommended; it will lower bacterial level.

Some of the isolates including *Enterobacter cloacae* and genera *Bacillus* and *Micrococcus*; have been reported to have beneficial health effect in fish and have been developed into fish probiotics [33, 34]. This may justify their occurrence in the gut of studied fish. However, further studies are needed to assess their efficacy as probiotics.

5. Conclusions and recommendations

This study has shown that farmed fish and their aquatic environments in Kirinyaga County harbor potentially pathogenic bacteria that can cause fish disease(s) especially under stressful intensive farming conditions. Moreover some of the bacteria have been documented to cause severe illness in humans; therefore may pose a serious public health hazard to consumers (in case fish and fish products are undercooked or consumed raw) and occupational hazards to fish harvesters (improper handling). Therefore, there is a need to implement optimal management and biosecurity programs at farm level. Further experimental infection challenge studies are recommended in order to evaluate the pathogenicity of single and/or multiple bacterial infections; so as to comprehend their significance on fish and human health.

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7. References

1. KMFRI. Kenya's Aquaculture Brief: Status, Trends, Challenges and Future Outlook. Kenya Marine and Fisheries Research Institute, Mombasa, Kenya, 2017, 12.
2. Mavuti SK, Waruiru RM, Mbuthia PG, Maina JG, Mbaria JM, Otieno RO. Prevalence of ecto-and endo-parasitic infections of farmed tilapia and catfish in Nyeri County, Kenya. Livestock Research for Rural Development. 2017; 29(6):55-9.
3. Maina KW, Mbuthia PG, Waruiru RM, Nzalawahe J, Murugami JW, Njagi LW *et al*. Risk factors associated with parasites of farmed fish in Kiambu County, Kenya. International Journal of Fisheries and Aquatic Studies. 2017; 5(4):217-223
4. Murugami JW, Waruiru RM, Mbuthia PG, Maina KW, Thaiyah AG, Mavuti SK *et al*. Helminth parasites of farmed fish and water birds in Kirinyaga County, Kenya. International Journal of Fisheries and Aquatic Studies. 2018; 6:6-12.
5. Mulei IR, Nyaga PN, Mbuthia PG, Waruiru RM, Njagi LW, Mwihia EW *et al*. Infectious pancreatic necrosis virus isolated from farmed rainbow trout and tilapia in Kenya is identical to European isolates. Journal of fish diseases. 2018; 41(8):1191-1200.
6. Mwihia E, Mbuthia P, Eriksen G, Gathumbi J, Maina J, Mutoloki S *et al*.. Occurrence and Levels of Aflatoxins in Fish Feeds and Their Potential Effects on Fish in Nyeri, Kenya. Toxins. 2018; 10(12):543.
7. Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R *et al*. Disease and health management in Asian aquaculture. Veterinary Parasitology. 2005; 132(3-4):249-272.
8. Öztürk RÇ, Altınok İ. Bacterial and viral fish diseases in Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 2014; 14(1):275-297.
9. Austin B, Austin DA. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Edn 6, Springer, New York, 2016, 732.
10. Maher M, Palmer R, Gannon F, Smith T. Relationship of a novel bacterial fish pathogen to Streptobacillus monilifomris and the fusobacteria group, based on 16S ribosomal RNA analysis. Systematic and Applied Microbiology. 1995; 18(1):79-84.
11. Akoll P, Mwanja WW. Fish health status, research and management in East Africa: past and present. African Journal of Aquatic Science. 2012; 37(2):117-129.
12. Serede IJ, Mutua BM, Raude JM. Calibration of Channel Roughness Coefficient for Thiba Main Canal Reach in Mwea Irrigation Scheme, Kenya. Hydrology. 2015; 3(6):55-65.
13. Naing L, Winn T, Rusli BN. 'Practical Issues in Calculating the Sample Size for Prevalence Studies'. Archives of Orofacial Sciences. 2006; 1:9-14.
14. Noga EJ. Fish Disease: Diagnosis and Treatment. Edn 2, Wiley Blackwell, Iowa, 2010, 538.
15. Roberts RJ. Fish Pathology. Edn 4, Wiley-Blackwell, Iowa, 2012, 581.
16. Bergey DH, Holt GH, Krieg NR, Peter HAS. Bergey's manual of determinative bacteriology. Edn 9, Lippincott Williams and Wilkins, 1994, 787.
17. Al-Harbi AH, Uddin MN. Seasonal variation in the intestinal bacterial flora of hybrid tilapia (Oreochromis niloticus × Oreochromis aureus) cultured in earthen ponds in Saudi Arabia. Aquaculture. 2004; 229(1-4):37-44.
18. Karimi RD. The bacterial flora of tilapia (*Oreochromis*

niloticus) and catfish (*Clarias gariepinus*) from earthen ponds in Sagana fish farm and Masinga dam. An MSc thesis in Infectious Disease Diagnosis (Kenyatta University), 2015.
19. Wamala SP, Mugimba KK, Mutoloki S, Evensen Ø, Mdegela R, Byarugaba DK *et al*. Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (*Nile tilapia*) and Clarias gariepinus (*African catfish*) in Uganda. Fisheries and Aquatic Sciences, 2018; 21(1):6.
20. Apun K, Yusof AM, Jugang K. Distribution of bacteria in tropical freshwater fish and ponds. International Journal of Environmental Health Research. 1999; 9(4):285-292.
21. Goegele H, Ruttmann E, Aranda-Michel J, Kafka R, Stelzmueller I, Hausdorfer H *et al*. Fatal endocarditis due to extended spectrum beta lactamase producing Klebsiella terrigena in a liver transplant recipient. Wiener Klinische Wochenschrift. 2007; 119(11):385-386
22. Shaikh MM, Morgan M. Sepsis caused by Raoultella terrigena. JRSM short reports. 2011; 2(6):1-3
23. Jeremić S, Jakić-Dimić D, Veljovic LJ. Citrobacter freundii as a cause of disease in fish. Acta veterinaria. 2003; 53(5-6):399-410.
24. Eissa NME, El-Ghiet EA, Shaheen AA, Abbass A. Characterization of Pseudomonas species isolated from tilapia "*Oreochromis niloticus*" in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Veterinaria. 2010; 5(2):116-121.
25. Shayo SD, Mwita CJ, Hosea KM. Virulence of Pseudomonas and Aeromonas bacteria recovered from Oreochromis niloticus (Perege) from Mtera hydropower Dam; Tanzania. Annals of Biological Research. 2012; 3:5157-5161.
26. Laith AR, Najiah M. Aeromonas hydrophila: antimicrobial susceptibility and histopathology of isolates from diseased catfish, Clarias gariepinus (Burchell). Journal of Aquaculture Research and Development. 2013; 5:215.
27. Cao H, Long X, Lu L, Yang X, Chen B. Citrobacter freundii: a causative agent for Tail Rot Disease in freshwater cultured Japanese Eel Anguilla japonica. The Israeli Journal of Aquaculture-Bamidgeh, 2016.
28. Nahar S, Rahman MM, Ahmed GU, Faruk MR. Isolation, identification, and characterization of Aeromonas hydrophila from juvenile farmed Pangasius (*Pangasianodon hypophthalmus*). International Journal of Fisheries and Aquatic Studies. 2016; 4(4):52-60.
29. Haenen OL, Evans JJ, Berthe F. Bacterial infections from aquatic species: potential for and prevention of contact zoonoses. Revue scientifique et technique (International Office of Epizootics). 2013; 32(2):497-507.
30. Adeshina I, Abdrahman SA, Yusuf AA. Occurrence of Klebsiella Species in Cultured African Catfish in Oyo State, South-West Nigeria. Nigerian Veterinary Journal. 2016; 37(1):24-31.
31. Ampofo JA, Clerk GC. Diversity of bacteria contaminants in tissues of fish cultured in organic waste-fertilized ponds: health implications. The Open Fish Science Journal, 2010;3:142-146
32. Bejerano Y, Sarig S, Horne MT, Roberts RJ. Mass mortalities in silver carp Hypophthalmichthys molitrix (Valenciennes) associated with bacterial infection following handling. Journal of Fish Diseases. 1979; 2:49-56.
33. Oggioni MR, Ciabattini A, Cuppone AM, Pozzi G. Bacillus spores for vaccine delivery. Vaccine. 2003; 21:S96-S101.
34. Capkin E, Altinok I. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease. Journal of Applied Microbiology. 2009; 106(4):1147-1153.