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Temperature and ph influence on bacterial pathogens infecting farmed Nile tilapia in aquaculture systems in Bungoma County, Kenya

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Abstract
Aquaculture production has been expanding in recent years through Governement support via financial and input subsidies in small scale farmers. However, fish farming just like other productive sectors face challenges which include fish diseases that constitute the largest cause of economic loss in aquaculture. The fish bacterial infections are caused by *Aeromonas, Edwardsiella, Flavobacterium, Francisella, Photobacterium, Piscirickettsia, Pseudomonas, Tenacibaculum, Vibrio, Yersinia, Lactococcus, Renibacterium, Streptococcus, Nocardia, Staphylococcus, Vagococcus* and *Weisella* species. This study focused on characterizing pathogenic bacteria present in aquaculture systems in Bungoma County. The sampled fish were transferred to a microbiology laboratory in Nairobi where they were subjected to bacterial analysis including microscopy, culture methods and biochemical tests. The study showed that temperature and pH were outside the recommended levels of fish culture production. Further, pathogenic bacteria recovered were *Vibrio vulnificus, Vibrio parahaemolyticus, Aeromonas hydrophila,* and *Pseudomonas aeruginosa* from Nile tilapia. *Vibrio vulnificus and Vibrio parahaemolyticus* were isolated from gills and were found to be significantly different (P<0.001) across the six sub counties. In fish ponds water, *Aeromonas hydrophila* were isolated and found to be significantly different (P<0.016) across the sub counties. In addition, *Streptococcus iniae* were isolated from fish feeds. Therefore, efforts should be made to reduce the presence of bacterial pathogens in aquaculture in order to increase fish yields.

Keywords: bacteria, diseases, fish pond, fish feeds and water quality

1. Introduction
In Kenya, the fisheries and aquaculture sub sector remains an important Department for food security, incomes and for trade development. Aquaculture production is practiced in regions endowed with a lot of water resources including springs, wetlands, rivers, streams and reservoirs. During production, various facilities are used including earthen ponds, cages, raceways, tanks, lockable ponds and recirculating systems. Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), Common carp (*Cyprinus carpio*) and Rainbow trout (*Oncorhynchus mykiss*) are the main fish species farmed [1-5]. The world per capita fish consumption is 20 Kg per person per year and this is due to increased production from aquaculture [6]. At national level, China, India, Viet Nam, Bangladesh, Egypt, Greece, Czech Republic, Hungary, Laos and Nepal produce more farmed fish than wild-caught fish [6].
To maximize the aquaculture opportunities that exist, the Government of Kenya has been supporting aquaculture production with a target to bridge a huge gap between supply and demand for fish in the Country [7]. However, fish farming just like other productive sectors faces challenges include fish diseases constitute the largest cause of economic loss [1, 8-9]. A global estimate by World Bank of losses in aquaculture due to diseases is approximately US$ 3 billion per annum [10]. Among the fish disease causing organisms, are 13 genera of bacteria which are implicated [11].
The genera include *Aeromonas, Edwardsiella, Flavobacterium, Francisella, Photobacterium, Piscirickettsia, Pseudomonas, Tenacibaculum, Vibrio, Yersinia, Lactococcus, Renibacterium, Streptococcus, Nocardia, Staphylococcus, Vagococcus* and *Weisella* [8, 11-14].
The diseases that occur in fish are as a result of the interaction between the farmed fish, potential pathogens and the fish ponds environment. Further, environmental factors such as temperature, oxygen content and pH influence bacterial infections in cultured fish [15-17].

Most warm water fish prefer at least 2 mg / l dissolved oxygen while an increase in stream temperature by 5ºC increases growth rate and adhesion capacity of the bacteria to fish tissues [18]. In Kenya, farmers are expected to keep records of water quality parameters and fish feeds should be regularly sampled and analysed [19]. This is because most pathogens in cultured fish are flora of the aquatic environment and they cause diseases under environmental stress [20]. *Vibrio vulnificus* have a growth optimum temperature at 37ºC, with a temperature range for growth of 8ºC, while *Aeromonas* spp. grow optimally at 28ºC with tolerant temperature range of 2°C to 45ºC [21- 23]. Suspended solids have been shown to affect fish health by physically abrading and clogging the gills, smothering eggs during incubation, abrading the skin and impairing visual feeding [24]. Ponds manured with animal excreta (turbidity), have very high counts of *Aeromonas* spp. [25].

The aim of this study was to determine the pathogenic bacteria present pond water, fish feeds and in Nile tilapia grown in aquaculture systems in Bungoma County. Bungoma County is located in Western Kenya and has an area of 3,032.2 Km^2. It lies between latitude 00º34'N and longitude of 34º34'E and altitude of between 1,200 and 1,800 meters above sea level. The mean annual temperature is about 23^{0}C with two rainy seasons; short and long with an average rainfall from 1200 mm to 1800 mm per annum. The County has three Agro-ecological zones: Lower Midland (LM 1), Lower Highland (LH) and Upper Highland (UH) [26]. In Bungoma County, the major farmed fish species is Nile tilapia. However, there are no documented studies on the kind of bacteria that are present and could infect it. The results from this study could be applied by extension officers to advice fish farmers in Bungoma County on bacterial pathogens that would infect their farmed fish and how best to manage their farms to minimize those infections. There is need for aquaculture practitioners' to have information on specific bacterial pathogens infecting individual fish species in order to put in place proper biosecurity systems to manage and control diseases in farmed fish.

2. Materials and Methods

2.1 Sampling procedures

2.1.1 Water quality parameters

At every fish pond sampled, five water quality parameters were assessed. The assessments were conducted using a portable auto sampler.

2.1.2 Fish

Nile tilapia was harvested from fish ponds using a seine net. After harvesting, organs such as mouth, scales, skin, gills, kidney, intestines and liver were removed and used to determine bacterial load. The mouth sampling was done by lifting the lower lip and inserting a sterile swab stick then rubbed inner walls. To obtain fish scale samples, a sterile scapel was used to scrap off the scales. For the skin samples, the fish scales were scrapped to expose the skin. The exposed skin was then layerly removed using a sterile scapel while holding with a sterile forcep. To obtain samples from the gills, intestines, kidney and liver, fish were dissected using a sterile scalpel and forceps to scoop the organs. All the organ samples except mouth samples were then cut into smaller pieces, crushed and 1 g taken and stored into a sterile bijou bottles. The bijou bottles were then packed in a cooler box and transferred to a Microbiology laboratory.

2.1.3 Pond water

At every fish pond, 100 ml of water was collected in sterile glass bottles, stoppered, enriched in alkaline peptone water and then ice-packed and delivered to the microbiology laboratory.

2.1.4 Fish feeds

One (1) Kg of fish feeds from each of the two feed millers who were the main suppliers operating in the county were purchased. The purchased fish feeds were transferred to the microbiology laboratory in Nairobi.

2.2 Analysis of fish samples

2.2.1 Stock solutions of fish samples

In the Microbiology laboratory, the cut tissue and mouth samples were individually and aseptically transferred into sterile 9 ml normal saline and were homogenised thoroughly to make 1:10 M/V stock solutions. The stock solutions (homogenates) were all incubated for 24 h at 37 ^{0}C. The homogenates were used for all the bacteriological tests [27]. The plating technique used was pour plate and was done in triplicate for every sample.

2.2.2 Total Viable Aerobic Colony Counts (TVACC)

From each solution of the samples, starting at the 10^0 (stock solution), 1 ml volumes were taken and each volume transferred to a sterile plate (petridish) and 20 ml of Tryptone soya agar (TSA) added to the plate and mixed well to ensure an even distribution of colonies. The agars were allowed to settle and inverted plates transferred to the incubator at 30 ^{0}C for 24 h. After 24 h, the number of colonies present on the plates was counted using a colony counter machine. The total viable aerobic colony counts were reported as colony forming units (cfu) per ml of the stock solutions.

2.2.3 Pathogenic bacteria

Vibrio vulnificus was isolated by taking 1 ml of the stock solution (10^0) transferred aseptically to flasks containing 9 ml of sterile salt-free alkaline peptone water (Himedia). The resulting homogenates were incubated at 30ºC for 24 h. Then a loopful was streaked on Thiosulphate – Citrate – Bile Salt (TCBS) agar and incubated for 24h at 30ºC. After 24 h, colonies were picked and subjected to TSA, Gram stain, TSI, catalase, methyl red and Voges-Proskauer, and hydrogen peroxide tests [28, 29].

The isolation of *Vibrio parahaemolyticus* was by the procedure as described by Letchumanan *et al.* [30]. One (1) ml of the stock solution (10^0) was taken and transferred aseptically to flasks containing 9 ml of sterile salt-free alkaline peptone water. The resulting homogenates were incubated at 30ºC for 24 h. Then a loopful was streaked on Thiosulphate – Citrate – Bile Salt (TCBS) agar and incubated for 24h at 30ºC. After 24 h, colonies were picked and subjected to TSA, Gram stain, TSI, catalase, methyl red and Voges-Proskauer, and hydrogen peroxide tests [28, 29].

Aeromonas hydrophila was isolated using the procedure as described by Al-Fatlawy and Al-Hadrawy [31]. One ml of the stock solution (10^0) was transferred aseptically to flasks containing 9 ml of sterile salt-free alkaline peptone water (Himedia, India). The resulting homogenates were incubated at 30ºC for 24 h. Then a loopful was streaked on TCBS agar

and incubated for 24h at 30ºC. After 24 h, colonies were subjected to further Gram stain, TSI, catalase, methyl red and Voges-Proskauer and hydrogen peroxide tests [28, 29].

Pseudomonas aeruginosa was isolated according to Austin and Austin [32]. One ml of the stock solution (10^0) was transferred aseptically to flasks containing 9 ml of sterile salt-free alkaline peptone water. The resulting homogenates were incubated at 30ºC for 24 h. Then a loopful was streaked on TCBS agar and incubated for 24 h at 30 ºC. After 24 h, colonies were subjected to TSA, Gram stain, TSI, methyl red, Voges-Proskauer and hydrogen peroxide tests [28, 29].

2.3 Analysis of water and fish feeds samples

In the laboratory, tenfold serial dilutions of water samples were prepared according to Torimiro *et al.* [33] Thereafter, the serial dilutions of water were subjected to TSA, Gram stain, TSI, catalase, methyl red and Voges-Proskauer, and hydrogen peroxide tests to enumerate and detect pathogenic bacteria as outlined for fish sample tests elsewhere as earlier described.

The purchased fish feeds were subjected to bacterial analysis by taking 1g of each fish feed and homogenizing into 9 ml of distilled sterile water using a sterile blender. Serial dilutions of the homogenates were made and then subjected to TSA, Gram stain, TSI, catalase, methyl red, Voges-Proskauer, and hydrogen peroxide tests to enumerate and detect pathogenic bacteria as outlined for fish sample tests above.

2.4 Data management and analysis

Data was entered into a Micro Soft Excel (Windows 10) spreadsheet and analyzed by SPSS Software. Confidence levels of 95%, 99% and 99.99% were considered significantly different. Kruskal-Wallis test was used to determine differences between and within the sub counties. Regression analysis was used to determine the effect of temperature, dissolved oxygen, electrical conductivity, pH and turbidity on the bacterial isolates in the farmed Nile tilapia.

3. Results

3.1 Water quality parameters

For all sampled fish ponds, it was found that there was a significant difference (P=0.001) in temperature of sampled fish pond water across the sub counties. While, among the sub counties, there was no significant difference (P=0.081) in dissolved oxygen across the different sub counties. All the conductivity levels among the six sub counties were found to be within recommended ranges for fish production. This implies that there was no stress exerted on farmed fish arising from conductivity. Further, the conductivity levels across the sub counties had no significant difference (P=0.677) in distribution. The recorded mean ranges 6.63 to 6.83 for pH are within allowable ranges for tilapia culture production. There was no significant difference (P= 0.928) in pH levels among the fish pond water across the sub counties. Besides, there was no significant difference (P=0.0110) across the sub counties.

Table 1: Means of water quality parameters of fish ponds

Sub County	Farms	Mean water quality parameters				
		Temperature (^{0}C)	Dissolved Oxygen (mg/l)	Conductivity (µs/ cm)	pH	Turbidity (ppm)
Bumula	10	30.35	2.80	88.20	6.72	15.00
Bungoma South	18	29.11	2.52	103.33	6.73	18.61
Bungoma West	25	26.74	3.04	83.64	6.63	15.40
Bungoma North	12	21.70	2.71	97.17	6.83	14.92
Mt. Elgon	5	23.64	2.85	91.00	6.71	17.40
Bungoma East	8	29.85	2.94	87.38	6.76	14.75

3.2 Total viable aerobic colony counts (TVACC)

Every fish sampled recorded varying levels of TVACC among the organs that were studied (Table 2). The fish mouths had higher TVACC than any other body parts. The high numbers are evident in all sub counties. The kidney and liver had the least varying from 3.20 TVACC in Bumula to 13.00 TVACC in Bungoma East.

Table 2: Means for total viable aerobic colony counts (TVACC) in Nile tilapia

Sub County	Farms	Mean (cfu/g)						
		Scales	Skin	Mouth	Gills	Intestine	Kidney	Liver
Bumula	10	119.50	77.70	138.30	79.90	103.80	3.20	11.60
Bungoma South	18	49.55	70.05	93.83	73.16	88.55	7.44	10.77
Bungoma West	25	121.76	85.36	134.96	112.44	90.48	9.40	9.92
Bungoma North	12	84.91	86.08	72.41	166.50	69.00	10.41	8.50
Mt. Elgon	5	42.40	88.40	104.80	76.00	3.40	10.00	7.00
Bungoma East	8	98.87	57.75	103.00	81.00	85.00	13.00	9.00

Table 3: Means for total viable aerobic colony counts (TVACC)

4.1. Results for TVACC in pond water		
Sub County	**Farms**	**Mean (cfu/ml)**
Bumula	10	60.30
Bungoma South	18	91.67
Bungoma West	25	128.44
Bungoma North	12	141.75
Mt. Elgon	5	189.00
Bungoma East	8	92.50
4.2. Results for TVACC in fish feeds		
Sub County	**Firms**	**Mean (cfu/g)**
Bungoma South	1	122
Bungoma West	1	97

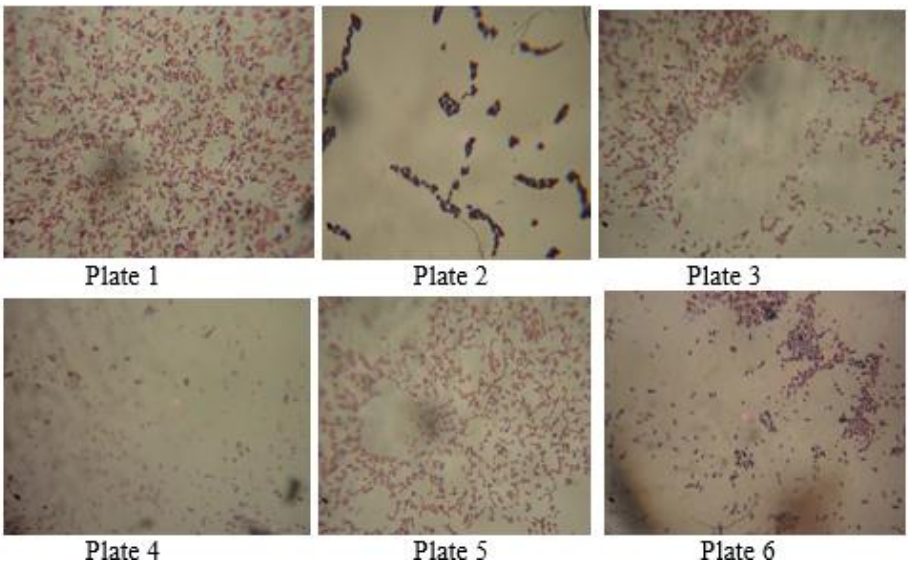
3.2 Pathogenic bacteria present in Nile tilapia

There were no pathogenic bacteria recovered in any of the organs in Nile tilapia sampled from farms in Bumula Sub County and Mt. Elgon Sub County. However, the other four sub counties (Bungoma South, Bungoma West, Bungoma North and Bungoma East) recorded presence of bacterial pathogens (Table 4).

Table 4: Means for pathogenic bacteria isolated from Nile tilapia

Sub County	Farms	Organ	Bacterial Species	Total colony counts	Mean (cfu/g)
Bumula	0	0	0		0.00
Bungoma South	5	Scale	*V. vulnificus*	10	2.00
Bungoma West	9	Skin	*V. vulnificus*	38	4.22
	6	Intestine	*P. aeruginosa*	6	1.00
Bungoma North	9	Gill	*V. vulnificus*	27	3.00
	6	Gill	*V. parahaemolyticus*	12	2.00
Mt Elgon	0	0	0		0.00
Bungoma East	5	Gill	*A. hydrophila*	3	0.60

In Bungoma South, the bacterial isolates were *Vibrio vulnificus* (Plate 1) averaging ($\bar{x}$ = 2.00cfu/g). However, there was no significant difference (P=0.975) in the distribution of the *V. vulnificus* isolated from the scales across the sub counties. In Bungoma West, *Vibrio vulnificus* (Plate 2) were present on the skin with a mean of 4.22cfu/g. There was no significant (P=0.992) difference in their distribution on the skin across the sub counties. *Vibrio vulnificus* (Plate 3) and *Vibrio parahaemolyticus* (Plate 4) were isolated in gills from sampled fish in Bungoma North. The two isolates *Vibrio vulnificus* and *Vibrio parahaemolyticus* were both found to have a significant difference (P< 0.001) in distribution among the Nile tilapia gills across the sub counties. In Bungoma East, *Aeromonas hydrophila* (Plate 5) were present in gills of selected Nile tilapia. The *Aeromonas hydrophila* isolated had a significant difference (P< 0.001) in distribution in the gills of tilapia sampled from different sub counties. *Pseudomonas aeruginosa* (Plate 6) isolated from intestines had a mean of 1.00cfu/g). Further, it was found that there was no significant difference (P=0.737) in the distribution of *P. aeruginosa* across the sub counties.



3.3 Pathogenic bacteria present in pond water and fish feeds

Aeromonas hydrophila (Plate 7-9) recovered from water sampled from Bumula, Bungoma South and Bungoma West sub counties respectively. The other three sub counties; Bungoma North, Mt. Elgon and Bungoma East had no pathogenic bacteria recovered. Whereas, *Streptococcus iniae* (Plate 10) was isolated from fish feeds sampled from Bungoma West Sub County but there was no bacterial pathogen that was isolated fish feeds sampled from Bungoma South Sub County (Table 5).

Table 5: Means for total colony counts of pathogenic bacteria in pond water and fish feeds

5.1. Total colony counts of pathogenic bacteria isolated from pond water				
Sub County	**Farms**	**Bacterial species**	**Total colony counts**	**Mean(cfu/ml)**
Bumula	2	*Aeromonas hydrophila*	17	8.50
Bungoma South	9	*Aeromonas hydrophila*	25	2.80
Bungoma West	17	*Aeromonas hydrophila*	20	1.18
5.2. Total colony counts of pathogenic bacteria isolated from fish feeds				
Sub County	**Firms**	**Bacterial species**		**Mean (cfu/g)**
Bungoma West	1	*Streptococcus iniae*	12	12

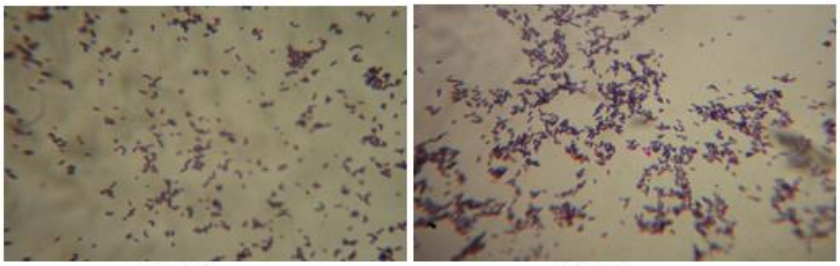


Plate 7 Plate 8

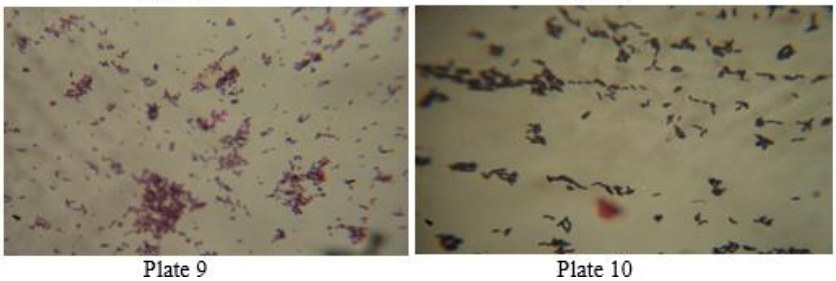


Plate 9 Plate 10

4. Discussion

4.1 Water quality parameters

The temperature ranges reported in the current study were from 20.04^{0}C to 32.63^{0}C implying that they were within the favourable ranges for the production of Nile tilapia. Required temperature in Kenya for production of fresh water fish is 22-32^{0}C for warm water and 14-17^{0}C for cold water fish. Further, the acceptable temperature ranges are from 15^{0}C-35^{0}C [34]. The optimal temperature for survival of *Vibrio vulnificus* is between 13^{0}C and 22^{0}C [35].

Dissolved oxygen levels were lowest at 1.78 mg/l and highest at 6.5 mg/l. There was only one fish pond that had less than 2 mg/ l of dissolved oxygen and could be attributed to pollution through runoffs during rainy season, floating artificial fish feeds that farmers use to feed their fish and manure applied by farmers to fertilize the fish ponds before restocking. The rest of the fish ponds sampled had dissolved oxygen levels above 2 mg/ l. The recommended dissolved oxygen levels should be above 2 mg/ l for the farmed Nile tilapia to survive favourably [18]. Furthermore, the required Dissolved oxygen in Kenya for production of fresh water fish is ≥3 mg/ l for warm water and ≥5 mg/ l for cold water [19].

Conductivity of water for fish ponds sampled ranged from 53 µs /cm to 372 µs /cm. In Kenya, recommended conductivity for production of fresh water fish is 2-10 µs/ cm for warm water as well as cold water [19]. However, conductivity of 9-400 µs / cm was observed in delta region, Nigeria [36]. The recorded conductivity ranges in the current study, falls within the recommended levels of conductivity for fresh water fish culture which is usually in the range of 20 to 1500 µs / cm [37]. The highest levels of conductivity were recorded in Bungoma South Sub County and could be attributed to key economic activity of sugarcane production. The sugarcane production is highly depended on the inorganic fertilizer whose ions would be washed into the fish pond waters. Conductivity hinders penetration of light in ponds making it difficult for aquatic habitat to receive the positive effect of light [16].

The lowest pH levels in the County were 5.94 and the appropriate pH for fish production is 6-9 [38-39]. The lower levels could be due to acidic soils characteristic of the two sub counties adjacent to each other and whose farmers rely on inorganic fertilizers for sugarcane production. During runoffs, the fertilizer remnants could be washed into fish ponds. Again the farmers in the two sub counties could be using the inorganic fertilizers instead of manure in fertilizing their fish pond. The recommended pH is 6.5-8.5 for both cold and warm water fish cultures in Kenya [19]. A range of 6.5-9.5 was documented for all ponds sampled in Niger delta in Nigeria [36]. The study currently recorded a pH range of 5.69-8.08 which are within the recommended level. The recommended levels for pH for suitable fish cultures are 4-11 [34].

Turbidity levels ranged from 8 ppm to 25 ppm. This could arise from the fact that all fish ponds are normally manured before stocking and restocking are done. It has to be noted that in Kenya, turbidity standards are 30-45 ppm [19] while other researchers have recommended ranges of 30-40 ppm [40] and 30-80 ppm and less than 12 ppm causing stress in cultured fish [41]. Therefore, according to the aforementioned recommendations, turbidity levels in fish ponds in Bungoma County were outside the required ranges at 12-80 ppm.

4.2 Total Viable Aerobic Colony Counts

Determination of AMCC in fish is very important to assess the extent of spoilage as well as contamination. High AMCC are normally due to either surface contamination or because of phenomenal growth of bacteria. Ranges of $10^2 – 10^7$ cfu/g for tropical fish species have previously been obtained [42, 43]. However ranges less than 10^6 cfu/g are recommended [44].

In the current study, Bumula and Bungoma West Sub Counties had the highest counts of TVACC ($\bar{x}$ >130cfu/g) in the mouths compared to the other four sub counties. This could be as a result of farmers in the two sub counties mostly relying on organic manure to fertilize their ponds before stocking with fingerlings. Manure is generally rich in microbial load and also provides a favourable environment for multiplication of any bacteria present in it. Generally, it was found that there was no significant (P=0.252) difference in distribution of TVACC in the mouths among the six sub counties.

Bungoma North Sub County had the highest TVACC ($\bar{x}$ =166.50 cfu/g) in gills among the six sub counties and the highest TVACC could be linked to use of inorganic fertilizer by the majority of farmers within the Sub County. Most farmers are maize growers and apply a lot of inorganic fertilizer. During runoffs, the inorganic fertilizer residues could be deposited in fish ponds. The fertilizer residues take up dissolved oxygen rendering competition to fish. Therefore, farmed fish takes in a lot of water for it to get sufficient oxygen thereby inhaling a lot of microbes. None-the-less, it

was found that distribution of TVACC in gills among the six sub counties was not significantly (P=0.381) different.

Mt. Elgon had extremely low TVACC ($\bar{x}$ =3.40cfu/g) whereas Bumula had very high TVACC ($\bar{x}$ =103.80cfu/g) in the intestines. The lowest mean in Mt. Elgon could be that the cool temperatures did not allow most of the microbes found on external organs namely scales, skin and the mouths to survive for long thereby penetrating the inner organs. The highest mean TVACC in Bumula could be attributed to the highest TVACC recorded for the mouth. This is because the mouth and the intestines are part of the digestive system and therefore, fish swallows microbes together with food materials allowing microbes to reach the intestines. However, it was found that there was no significant (P=0.307) difference in distribution of intestine TVACC among the sub counties.

The lowest mean of TVACC in Bungoma East may be attributed to the fact that most farmers in the Sub County rely on springs and boreholes to supply water to fish ponds. In most cases, the borehole water is treated minimizing the microbial load whereas natural spring water has low level of microbial load unless contaminated down streams. Further, it was noted that there was no significant (P=0.067) difference in distribution of TVACC on the skin across the different sub counties.

Generally, kidneys and livers had very low ranges ($\bar{x}$ ≥3.20cfu/g ≤ 13.00cfu/g) of TVACC. This could be attributed to the fact that the two organs are part of the excretory system and most of the TVACC had been removed from the body of fish. Besides, it was found that the kidneys had significant difference (P=0.015) in distribution while the liver had no significant difference (P=0.903) in distribution across the different sub counties.

4.3 Pathogenic bacteria

The recoveries of *Vibrio vulnificus, Vibrio parahaemolyticus* and *Aeromonas hydrophila* from scales, skin and gills could be attributed to the fact that three organs (scales, skin, and gills) are organs that come into contact with water (external environment). Once in contact, pathogenic bacteria would attach themselves to the skin and scales and also on gills during respiration. Besides, the bacteria could have been present in fingerlings originally and remained in the organs or naturally present in pond water. The presence of *Vibrio* spp. is in line with their occurrence in Nile tilapia grown in aquaculture units in Alvarado in Mexico [45]. The presence of *Pseudomonas aeruginosa* could be attributed to possible presence in pond water sediment during feeding and breeding. Furthermore, *Pseudomonas* spp. was present in Nile tilapia culture pond sediment in Hai Duong Province in Vietnam [46].

Aeromonas hydrophila isolated from pond water in Bumula, Bungoma South and Bungoma West Sub Counties could be attributed to cross contamination. During rainy season, runoffs could spread the bacteria. *Aeromonas* spp. was observed in water drawn from some concrete ponds in delta region in Nigeria [36].

Though during this study there were no recoveries of *Streptococcus iniae* from the sampled fish and pond water, the bacteria was isolated from fish feeds sampled from Bungoma West Sub County. Therefore, fish feeds could be a source of pond water contamination by *S. iniae*. Fish feeds are able to introduce a wide variety of micro-organisms into the fish ponds [47].

5. Conclusion

It was found that only temperatures and pH were outside the recommended levels of fish culture production. Another notable observation in this study was that when the total viable aerobic colony counts were more than 51cfu/ g, there was a likelihood of the presence of pathogenic bacteria in farmed Nile tilapia. *Vibrio vulnificus* and *V. parahaemolyticus* were observed in TVACC above 72 cfu/ g; *A. hydrophila* when TVACC more than 51 cfu/ g; and finally *P. aeruginosa* when TVACC was more than 51 cfu/ g; while *P. aeruginosa* was found when TVACC were in excess of 100 cfu/g. Therefore, the TVACC could be used as a monitoring indicator so that when TVACC is beyond 74.33cfu/ g, then measures should be taken to prevent potential pathogenic bacteria from multiplying to cause diseases in farmed Nile tilapia.

The pathogenic bacteria, *V. vulnificus*, *V. parahaemolyticus*, *A. hydrophila* and *P. aeruginosa* identified in this study could be responsible for retarded growth of fish observed in farmed Nile tilapia. It is possible that *Aeromonas hydrophila* in pond water and *S. iniae* in fish feeds could be source of contamination.

6. Recommendation

Efforts should be made to reduce the presence of bacterial pathogens in aquaculture in order to increase fish yields. In addition, fish farmers should be trained on fish husbandry so that they are able to monitor water quality parameters and biosecurity measures instituted against any opportunistic contamination.

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