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Prevalence of potential pathogenic and zoonotic aerobic bacteria in wild and farmed *Oreochromis jipe, Oreochromis niloticus* and source water in Taita-Taveta County, Kenya

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Abstract
Bacterial infections can cause latent, low to high mortalities in aquaculture farms and wild fish while some bacteria may be zoonotic. This study isolated, characterized and identify potential pathogenic and zoonotic aerobic bacteria in farmed and wild *Oreochromis jipe*, *O. niloticus*, their hybrids and culture water source from Taita-Taveta County. One hundred and eleven apparently healthy-appearing fish consisting of 67 *O. jipe*, 34 *O. niloticus* and 10 hybrids and nine water (7 farms and 2 Lake Jipe) samples were processed. Samples were aseptically collected from each fish, namely, skin and kidney swabs; gills and intestinal tissues. Conventional culture, biochemical and Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry tests were done to identify isolated bacteria. A total of 596 bacterial isolates representing 22 genera were recovered from 444 fish organs and 9 water samples. Of these, the highest numbers were from the gills at 29.7% followed by skin 29.6%, intestines 24.4%, kidney 11.8% and the least from water at 4.7%. Potential fish pathogens were *Bacillus cereus* (8%), *Aeromonas veronii* (8%), *Aeromonas hydrophila* (5%), *Lysinibacillus fusiformis* (5%), *Acinetobacter Johnsonii* (4%) and *Acinetobacter solii* (4%). Potential pathogenic and zoonotic bacteria isolated were *Staphylococcus lentus* 17.9% (5/28), *Aeromonas hydrophila* 10.7% (3/28) and *Aeromonas hormaechei* 7.1% (2/28). From the sampling site; 28.4% (169/596) of the total isolates were collected from Taveta, then Mwatate with 26.9% (160/596), Mkwajuni 16.8%, (98/596), Kenya Wildlife Service 7.0% (42/596), Wundanyi 6.7% (40/596), Voi 6.6% (39/596) and the inlet of the Lake Jipe had a 3.2% (19/596) and from the water samples 4.7% (28/596).
These findings confirm that fish in aquaculture farms, wild fish and water used for aquaculture harbors potentially pathogenic and zoonotic bacteria which may cause fish diseases and pose public health risks. Extension officers and farmers need awareness on mitigation measures against these pathogens.

Keywords: *Aeromonas hydrophila*, aquaculture, fish pathogens, prevalence, wild fish, Zoonosis

1. Introduction
Global fish production has been projected to be 178 million tonnes with aggregate worthy 406 billion US dollars in 2018 [1]. Of them, aquaculture accounts for 88 million tonnes which is 49% (88/178) of the total production valued at USD $265 billion. Africa accounts for 1.92% of the total aquaculture production with Egypt (67.62%) and Nigeria (11.12%) being the main contributors [1]. Kenya was placed 4th largest producer of aquaculture in Africa, with a contribution of 0.2% in the period 2009-2013 [3] and 7th largest in 2020 with 0.9% contribution [2]. In the quest to improve aquaculture production in Kenya, the Government launched the Economic Stimulus Programme (ESP) which was a large-scale aquaculture support program during the period 2009-2013, aimed at capacity building of fish farmers [4, 5]. In 2010, 27,000 fish ponds were constructed nationally, 650 in Taita Taveta county through the ESP program, but only 380 fish ponds are still actively productive while 270 are dormant because fish farmers feel abandoned because of lack of sustainable technical support and the high cost

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of commercial fish feeds [4].

Taita-Taveta County is located on Kenya's coast. The county depends on both capture fisheries and aquaculture as the main source of fish [5]. Capture fisheries are derived from lake Chala and lake Jipe. The main fish species of these two lakes include *Oreochromis hunter* of Lake Chala; and *Oreochromis niloticus*, *Clarias gariepinus* and *Oreochromis jipe* from Lake Jipe [5]. The lake Jipe produces an average of 3-4 metric tonnes monthly of Tilapia and Clarias fish species, this is a key livelihood activity for the local communities surrounding the lake and a source of fish for nearby Taveta, Voi, Wundanyi and Mwatate towns. It employs approximately 200 fishermen directly and supports over 20,000 livelihoods indirectly in Kenya and Tanzania [5]. Hence, identifying the pathogens that limit aquaculture and wild fish productivity will enhance the livelihood of many communities.

In the county, majority of fish farmers rear *Oreochromis niloticus* (90%) and *Clarias gariepinus* (10%). Most farmers use earthen ponds as the main units of culture, followed by liner ponds and the least use concrete fishponds [5]. The use of earthen ponds predisposes the fish to health challenges since they have been associated with heavy worm burdens and bacterial pathogens [6]. In 2019, Kenya Marine and Fisheries Research Institute (KMFRI) and County government of Taita-Taveta launched an initiative to restore the endangered *Oreochromis jipe* and prevent its extinction [7]. The reason for this diminished number is not clear [8]. Could pathogens infections, especially bacterial ones be a reason? This was investigated.

Oreochromis jipe is an endangered fish species that is threatened by extinction, according to [7] this fish is mainly found in Lake Jipe and "Nyumba ya Mungu" in Tanzania, It is on the Red List Assessment of Critically Endangered Species of the International Union for Conservation of Nature (IUCN) . The introduction of *Oreochromis niloticus* into the lake Jipe was examined against potential threat to biodiversity, actual stocks, and future potential fisheries yields of *O. jipe* and would have led to its extinction [9]. There are at least twelve (12) species of fish belonging to five families found in Lake Jipe. These include the endemic tilapia; *Oreochromis jipe*, *O. esculentus*, *Oreochromis niloticus*; mudfish (*Clarias mozambicas*), *Astatotilapia bloyeti*, *Petersius tangensis* and the sardine *Rastineobola argentae* [10, 11].

Unsustainable anthropogenic activities in lake Jipe ecosystem catchment have brought the species to endangered status while causing decline of some other fish species [10, 12]. Despite this imminent danger, there have been no studies conducted to determine the health status of both wild and farmed fish establishments [8].

Biosecurity status in fish farms is unstable with the current increased demand foreign trading environment caused by globalization, intensification in fish farming and climate change [13]. According to the World Organization of Animal Health (WOAH) [14] and Aquatic Animal Health Code [15], there is need for surveillance to be carried out with the following objectives (OIE, 2021-2025):(1) Declaring the absence of a disease; (2) Establishing events that necessitate notification; (3) Regulating the incidence or prevalence of endemic diseases, including changes in incidence or prevalence, in order to: (i) Give information on disease control in Kenya ; and (ii) Provide trading partners with relevant disease occurrence information for both qualitative and quantitative risk assessments.

Most fish farmers are unfamiliar with proper health management practices, so they continue to suffer from continuous losses as a result of various pathogens, particularly bacteria, in their fish farms [16]. Therefore, this study will provide essential information to fish farmers in Taita-Taveta County and elsewhere on potential bacterial pathogens causing diseases likely to affect the *Oreochromis* fish species and possibly cross to humans, sensitize them on good fish farm husbandry to ensure sustainable aquaculture activities and production of healthy fish.

The importance of aquaculture is shifting, thus the need for more surveillance for fish diseases, not only during production, but also for its contribution to development of antimicrobial resistance (AMR) through misuse of antibiotic treatments and its zoonotic potential. This can occur after direct skin contact (when humans have injured skin), with sick fish, culture water and when fish are consumed raw or people are immunocompromised [17, 18].

With an increasing demand for healthier foods consumption especially fish and fisheries products, zoonotic bacterial pathogens have become a major issue of concern worldwide. However; research on fish derived diseases are still inadequate [18]. Therefore, this study aims to find out the prevalence of the potential pathogenic and zoonotic bacteria associated with wild and farmed *Oreochromis* species in Taita-Taveta County.

2. Materials and Methods

2.1 Study area

The study was done along the shores of Lake Jipe (particularly at the inlet near River Lumi, Kachero beach, KWS station and at the outlet near River Ruvu) and *Oreochromis Jipe* farms in Voi, Mwatate, Wundanyi and Taveta [19].

2.2 Sampling procedure and sample size determination

Five farms within Voi (n=1), Mwatate (n=1), Wundanyi (n=1) and Taveta (n=2) were selected for sampling in sub-counties in Taita-Taveta County. They were purposively selected because they were active fish farms rearing *Oreochromis jipe*. Similarly, the 4 sites within the lake were purposively selected based on accessibility. The sample size was calculated using the formula by Bartlett *et al*. [20]: where $n=\frac{z^2 p\,(1-p)}{d^2}$ n is the sample size, Z is the Z statistic for confidence level of (1.96 for 95%), P is the assumed prevalence level which is equal to (50%) and d is the precision, which is equal to 5% (0.05): This calculation gave a sample size of 384 (Three hundred and eighty four). In the 9 sites, a total of 111 healthy-looking fish (67 *O. jipe*, 34 *O. niloticus* and 10 hybrids) and culture water were collected. From each fish, 4 organs (skin, gills, intestines and kidney) samples were taken. A total of 444 samples were collected for bacteriological examination.

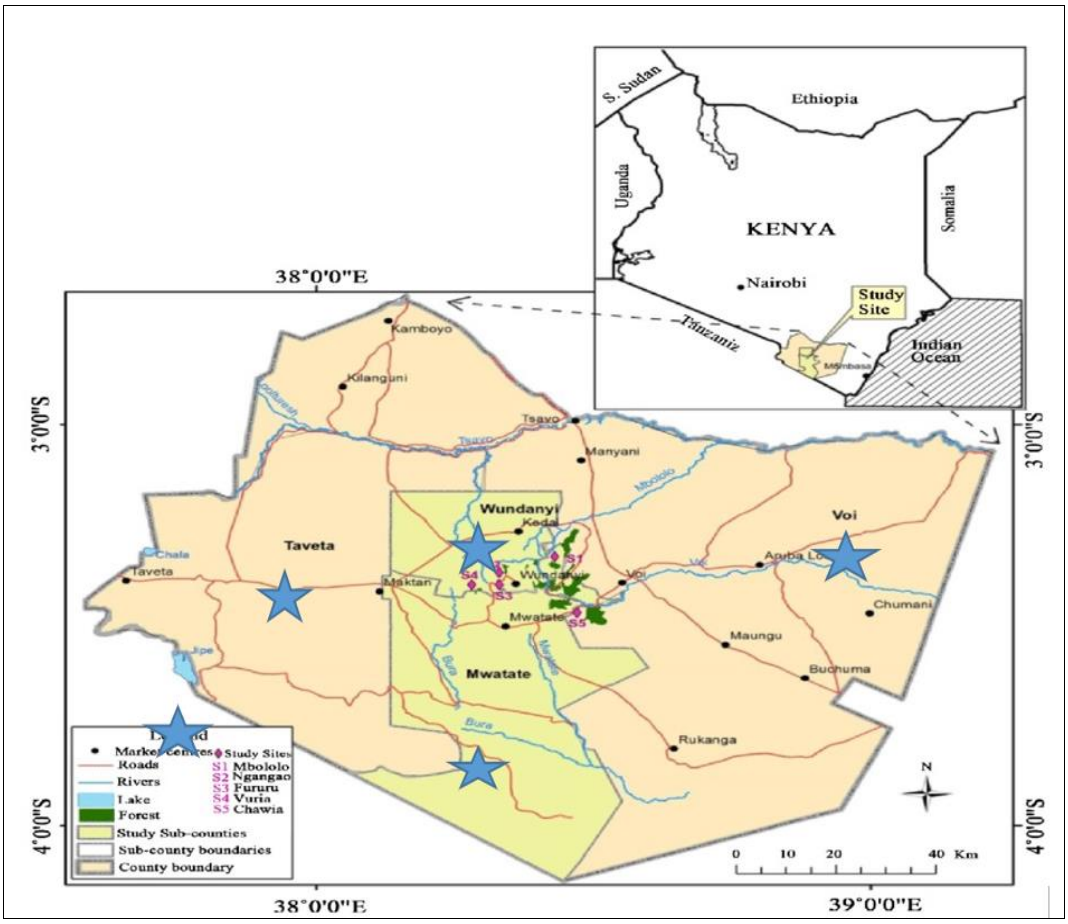

Fig 1: Map of County Government of Taita-Taveta showing the Lake Jipe and respective Sub-Counties [19].

2.3. Study animals

Grow-out to table size (200g) tilapia jipe (*Oreochromis jipe,* Fig 2 A), locally known as "Asilia", *Oreochromis niloticus* (Fig 2 B) locally known as *"Kuka"* and their hybrids (Fig 2 C) were sampled from the lake and randomly selected active fish farms in Taita-Taveta County. *Oreochromis jipe* has steep and nearly straight upper head profile, 34 scales along the lateral line and some vertical stripes on the caudal fin [21].

Fig 2: Shows *Oreochromis jibe* (A), *Oreochromis niloticus* (B) and their hybrid (C) that were sampled for the study

2.4 Ethical clearance

Research approvals were granted by Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (REF: FVM BAUEC/2023/419) and National Commission for Science, Technology (NACOSTI) REF: (549523). An informed verbal consent was asked from fish farmers before field sampling.

2.6 Necropsy and organ sampling

Post-mortem examination was done as described by Roberts [22]. Prior surface decontamination of the working benches was done using 70% alcohol. The dissecting instruments were initially autoclaved at 121ºC for 15 minutes. For re-use, the instruments were immersed in 70% ethanol for 2 minutes followed by flame sterilization. The fish were killed by giving a hard hit on the cranium. Externally the fish were examination for gross lesions such as wounds, hemorrhage, ulceration, and erosion. Each fish was laid on its lateral surface on the prepared bench.

Sterile cotton swabs were used to get samples from the skin using a sterilized aluminum metal plate with square inch fenestration was used as a guide. The operculum was removed to reveal the gills, after which a portion of the gills was excised. The external surface and organs of the fish were cleansed with 70% alcohol prior to opening of the body cavity [23]. The fish were then dissected to expose abdominal organs. A section of the intestine and kidney swabs was then taken aseptically. A portion of the intestine and kidney swabs were

then taken aseptically and separately placed in bijou bottles containing Stuart's transport media, labeled, and put in a cooler box with ice. As soon as possible, all samples were transported to the bacteriology laboratory in the department of Veterinary Pathology, Microbiology and Parasitology for further analysis.

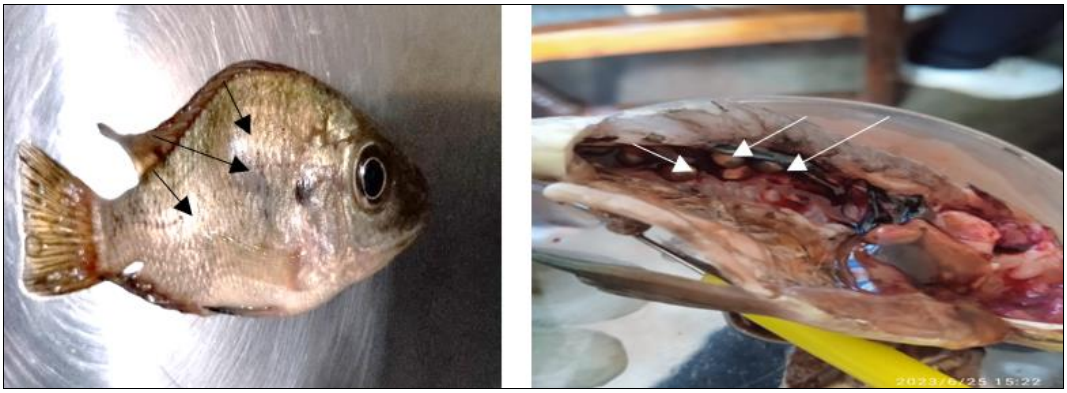

Fig 3: A Sampled *Oreochromis niloticus* showing abnormal spinal cord (black arrows) and B *Oreochromis jipe* showing infestation of kidney with multiple nodules (white arrows).

2.7 Processing of samples and bacteria isolation

The sampled organs (gills and intestines) for each individual fish were pulverized using sterile physiological saline, a homogenate of the then made using sterile pestle and mortar. The homogenate plus the kidney swabs were incubated overnight at 37 °C in freshly prepared alkaline peptone water (APW; pH 8.4) [24].

An inoculum was sub-cultured by streaking aseptically onto plates containing blood agar and MacConkey agar using a flame sterilized nichrome wire loop. According to Sanders [25], the plates were subsequently incubated aerobically at 37 ^{0} C and at the room temperature, after 24-48 hours of incubation, during which time growth occurs. Examination on growth and colonony morphology of the bacteria were done on the plates and documented. To obtain pure colonies, distinct colonies were sub-cultured.

2.8 Identification of isolated bacteria

Following Austin and Austin [26] and Bergeys Manual of Determinative Bacteriology [27], the isolates were identified using colony morphology, Gram staining characteristics, and conventional biochemical tests and further bacterial identification was done at National Public Health Laboratory and at the International Livestock Research Institute (ILRI) Kabete, using Matrix Assisted Laser Desorption/Ionization – Time of Flight technique (MALDI-TOF MS).

2.9 Data management and analysis

The collected raw data was put into a Microsoft Excel data sheet and analyzed with the SPSS-22.0 statistical software. Calculating proportions and frequencies of all the variables was done using descriptive statistics.

3. Results and Discussion

3.1 Colony morphology characterizations of the isolates

Depending on the colony morphology on different culture medium, some isolates were identified. Figure 3 (A) is *Aeromonas hydrophila* colonies which are off white circular, β-haemolytic on blood agar plate , 3(B) is *Pseudomonas putida* colonies which are off white, haemolytic colonies on blood agar, 3(C) is *Staphylococcus lentus* colonies which produces golden yellow color on Mannitol Salt Agar, 3 (D) is *Streptococcus scuiri* colonies which are pinpoint, off white colonies on blood agar, (E) is *Klebsiella variicola* which produces red mucoid colonies on MacConkey media, (F) is *Klebsiella pneumonia* which forms pink mucoid lactose fermenting colonies on MacConkey media, (G) is *Bacillus cereus* colonies which produces dry, giant and hemolytic colonies on blood agar, Fig 3(H) is *Lysinibacillus fusiformis* colonies has a swarming characteristic following spot inoculation on blood agar, and lastly (1) is *Macrococcus luteus* colonies which forms bright yellow pigmentations on Tryptose Soy Agar (TSA).

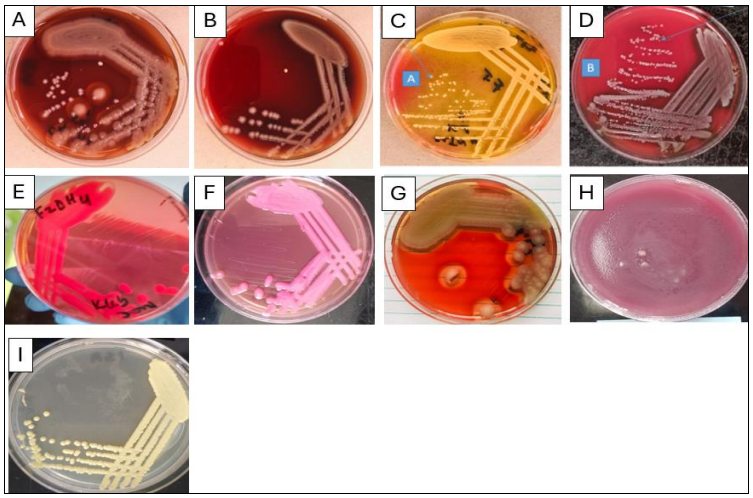

Fig 4: Plate showing some bacteria isolates in different culture media

3.2 Prevalence of bacteria isolated per fish organ and water sample

Based on MALDI-TOF MS results, five hundred and ninety-six bacteria were recovered from 111 apparently healthy fish. The bacteria species were isolated from gills at 29.7% (177/596) followed by skin 29.6% (176/596), intestines 24.4% (145/596), kidney 11.8% (70/596) and from water at 4.5% (28/596).

Among the bacteria species the most prevalent bacteria were *Staphylococcus* (19.9%) (118/596), *Bacillus* (17.7%) (106/596), *Acinetobacterium* (16.4%) (98/595), *Aeromonas* (15.6%) (93/596) and *Pseudomonas* (5%) (30/596).

3.3 Prevalence of bacterial isolates from fish and water samples

Out of 596 isolates, 568 (five hundred and sixty-eight) were recovered from fish organs and 28 (twenty-eight) from the water samples. A total of 54 bacteria species in 22 genera were recovered. Of these, the Gram negative bacterial isolates were 313 (52.6%) and the gram positive were 283 (47.3%). There were more gram negative bacteria compared to gram positive bacteria isolates which agreed with Dissasa *et al*. [24] in a study on live, processed fish and water samples from three Ethiopian rift valley lakes who isolated 154 bacterial species, 51(33%) gram-positive and 103 (67%) gram-negative isolates. Wanja *et al*. [23] also identified more gram negative bacteria compared to the gram positive bacteria although at varying percentage. From the current study, Gram positive identified were in the family Bacillus, *Micrococcus, Microbacterium, Macrococcus, Kytococcus, Exiguobacterium, Metabacillus, Streptococcus, Priesti, Corynebacterium and* gram negative identified were; *Aeromonas, Acinetobacter, Escherichia coli, Pseudomonas, Klebsiella, Laclercia, Pontoea, Empedobacter, Enterbobacter, Citrobacter, Stenotrophoimonas*

The most common genus identified include *Staphylococcus* (19.9%) (118/596) *Bacillus* (17.7%) (10/596), *Acinetobacterium* (16.4%) (98/596), *Aeromonas* (15.6%) (93/596), *Pseudomona* (5%) (30/596) and *Escherichia coli* (19/596) 3.18%. Others include *Streptococcus, Macrococcus, lysinibacillus, Klebsiella, Escherichia Coli, Enterobacterium, Citrobacter, Pontoea ananatia, Kytococcus, lactococcus, Exiguobacterium, Chryseobacterium, leclercia, Corynebacterium, Priestia, Empedobacter* were recovered (Table 1). These finding agrees with those of Charo *et al*. [28] who isolated *Aeromonas* at 32% as the most common gram negative bacteria and *Bacillus* 29% as the most prevalent gram positive bacteria. Bacteria of the genus *flavobacterium* were not isolated from the current study which were isolated by Charo. The isolation of *Escherichia coli* in the current study indicates possible fecal contamination of from the sampling sites, as reported previously by Dissasa [24].

Gills had 29.7% (177/596) followed by skin 29.6% (176/596), intestines 24.4% (145/596) kidney 11.8% (70/596) and the least from water at 4.5% (28/596). High concentration of bacteria load in skin was observed by Wanja *et al*. [29]. Isolation of bacteria in the kidney occurs when bacteria pathogens defeats the fish's defense mechanisms or in case of case of infections of hematogenous fish organs as described by Pech *et al*. [30]. High concentration of the isolates was found in the skin, gills and intestine and the least from the kidney. Bacteria from *Enterobacteriaceae* family are were isolated from this study, although many of them are usually normal fish microbiota, some like *E. coli, Klebsiella penumoniae* and *Pseudomonas* spp are considered zoonotic and have serious consequences to public health [30].

Table 1: Pathogenic and Zoonotic Bacteria Isolated from fish and water samples

Bacteria genera	Bacteria species	Bacteria isolates	Percentage	Pathogenic	Zoonotic	References
Staphylococcus species	*Staphylococcus lentus*	77	12.9	+	-	[32]
	Staphylococcus equorum	6	0.1	Normal	-	[33]
	Staphylococcus capitis	1	0.17	-	-	New
	Staphylococcus haemolyticus	1	0.17	+	+	[34, 35],
	Staphylococcus kloosii	1	0.17	-	-	New
	Staphylococcus variabilis	1	0.17	-	-	New
	Staphylococcus warneri	1	0.17	-	-	New
	Stapylococclus epidermids	20	3.35	+/Normal	Normal	[36]
	Staphylococcus ureilyticus	10	1.67	-	-	New
Bacillus species	*Bacillus cereus*	48	8.05	+	+	[37]
	Bacillus pumilus	18	3.02	Normal	Normal	[37, 41]
	Bacillus thuringiensis	2	0.33	Normal	Normal	[38, 37]
	Bacillus subtilis	1	0.17	-	+	[37]
	Cytobacillus kochi	1	0.17	-	-	New
	Peribacillus simplex	5	0.84	-	-	New
	Lysinibacillus fusiformis	29	4.86	-	-	[39]
Aeromonas species	*Aeromonas veronii*	45	7.55	+	+	[40, 41, 42, 36, 43]
	Aeromonas hydrophilla	31	5.2	+	+	[40, 44]
	Aeromonas spp	1	0.17	+	-	[40]
	Aeromonas hormaechei	16	2.68	-	-	New
Acinetobacter species	*Acinetobacter courvalinii*	2	0.33	-	-	New
	Acinetobacter schindleri	2	0.33	-	-	New
	Acinetobacter modesta	8	1.34	Not	-	New
	Acinetobacter johnsonii	26	4.36	+	+	[45]
	Acinetobacter solii	26	4.36	-	-	New
	Acinetobacter lwoffii	20	3.35	+	+	[36, 46]
	Acinetobacter variabilis	13	2.18	Normal	Normal	New
	Acinetobacter gyllenbergii	1	0.17	Emerging+	+	Emerging
Escherichia coli	*Escherichia coli*	19	3.18	-	+	[47, 48]

Pseudomonas species	*Pseudomonas fulva*	17	2.85	-	+	[49]
	Pseudomonas stutzeri	8	1.34	-	-	New
	Pseudomonas putida	5	0.8	+	+	[40, 50]
Micrococcus species	*Micrococcus leteus*	8	1.34	Normal	Normal	[31, 51]
Microbacterium oleivoran	*Microcobacterium oleivoran*	16	2.68	-	-	New
Macrococcus species	*Macrococcus caseolyticus*	10	2.01	-	-	[52]
Priestia species	*Priestia megaterium*	22	3.69	Plant pathogen	-	[53]
Klebsiella species	*Klebsiella pneumonia*	10	1.67	+	+	[40, 33],
	Klebsiella variicola	4	0.67	+	+	[40]
Streptococcis species	*Streptococcus sciuri*	10	1.68	-	-	New
Corynebacterium species	*Corynebacterium jeikeim*	7	1.17	-	-	New
	Microbacterium oleivorans	14	2.35	-	-	New
Leclercia species	*Leclercia adecarboxylata*	7	1.17	-	+	[54]
Pantoea species	*Pantoea ananatia*	5	0.84	-	-	New
Empedobacter species	*Enterobacter cloacae*	4	0.67	+	+	[55, 56, 57]
	Empedobacter falseni Empedobacter tilapiae	2	0.33	+	+	[58]
Enterobacter species	*Enterobacter bugandensis*	3	0.5	+	+ New	[55]
Exiguobacterium species	*Exiguobacterium indicum*	4	0.67	-	-	Not reported fish pathogen
Chryseobacterium species	*Chryseobacterium gambrini*	2	0.33	-	-	New
Citrobacter species	*Citrobacter brakii*	2	0.33	+	+	[51, 55, 43]
Kytococcus species	*Kytococcus sedentarius*	1	0.17	-	-	New
Lactococcus species	*Lactococcus garvieae*	1	0.17	+	+ Emerging	[59, 60, 42]
Stenotrophoimonas species	*Stenotrophoimonas maltophila*	1	0.17	+	+	[61]
metabacillus species	*metabacillus indicus*	1	0.17	-	-	New
		596		21+ , 13, normal, 4 emerging, I plant pathogen	30+ ,2 emerging	

Key: += (pathogenic or zoonotic), normal=commensal bacteria, emerging =newly isolated bacteria, new=bacteria isolated for the first time

3.4 Prevalence of bacteria based on sampling sites

From the sampling site; 28.4% (169/596) of the total isolates were collected from Taveta, then Mwatate with 26.9% (160/596), Mkwajuni 16.8% (98/595), KWS 7.0% (42/596), Wundanyi 6.7% (40/595), Voi 6.6% (39/596) and the inlet had a 3.2% (19/596) and from the water samples 4.7% (28/596) (Figure 5). Bacteria isolated that were common in the Lake, Farms and Water samples, include; *Staphylococcus lentus, Bacillus cereus, Aeromonas hydrophilla, Lysinibacillus fusiformis, Priestia megaterium,* and *Acinetobacter johnsonii*. Roh and Kannimuthi [63]. confirmed the possibility of disease transmission between the wild and the culture systems in aquaculture systems. Most of the farmers acquire brooders especially of the *Oreochromis niloticus* from the lake Jipe because of lack of a certified fish hatchery within the county and this would potentially lead to disease outbreaks.

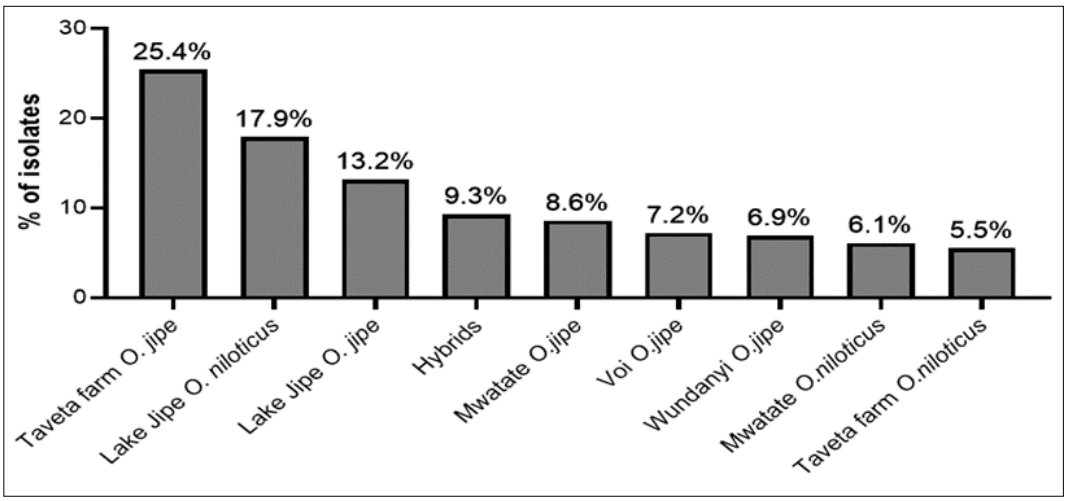

Fig 5: Percentage of bacteria sampled and sampling site

3.5 Prevalence of potentially pathogenic bacteria isolated in the fish samples and water

Of the bacteria species isolated some of them were identified as potential fish pathogens which included ; *Bacillus cereus* (8%) (48/596) *Aeromonas veronii* (8%) (45/596), *Aeromonas hydrophilla* (5%) (31/596), *Lysinibacillus fusiformis* (5%) (29/596), *Acinetobacter Johnsonii* (4%) (26/596), *Acinetobacter solii* (4%) (26/596), *Priestia megaterium* (4%), *Acinetobacter iwoffii* (3%) (20/596), *Escherichia coli* (3) (19/596), *Pseudomonas fulva* (3) (17/596), *Klebsiella pneumonia* (2) (10/596) *Streptococcus sciuri* (2) and (10/596) *Empedobacter tilapiae* (2%) (10/596). Others include

Corynebacterium jeikeim, Lcelercia adecarboxylata, Pseudomonas putida, Enterobacter cloacea, and Exiguobacterium (Table 1). Chitambo *et al.* [62] identified bacteria of the family *Acinetobacter, citrobacter, corynebacter, pseudomonas, micrococcus and strepotoccus* as the pathogenic fish bacteria at varying intensity unlike this study.

Potential zoonotic bacteria species isolated were; *Bacillus cereus* (8.05%), *Bacillus subtilis* (0.17%), *lysinibacillus fusiformis* (4.85%), *Aeromonas veronii* (7.55%), *Acinetobacter johnsolii* (4.36%), *Acinetobacter solii* (4.36%), *Acinetobacter iwofii* (4.36%) *Escherichia coli* (3.18%), *Pseudomonas fulva (*2.85%), *Pseudomonas putida* (0.8%), *Klebsiella pneumonia* (1.67%), *Klebsiella variicola* (0.67%), *Leclercia adecarboxylata* (1.17%), *Enterobacter cloacea* (0.67%), *Empedobacter falseni* (0.5%), and *Cryseobacterium gambrini* (0.33%), *Kytococcus sedentarius* (0.17%), *Lactococcus garvieae* (0.17%), *Stenotrophoimonas maltophila* (0.17%) and *Metabacillus indicus* (0.17%). Chitambo *et al.* [62] identified bacteria of the family *Aeromonas, Bacillus, Escherichia coli, Klebsiella, Lactococcus, Staphylococcus* and *Streptococcus* as potential zoonotic bacteria isolated from *Oreochromis niloticus* but did not report 8 other genera in this study. Ogala *et al.* [31] isolated *Streptococcus, Aeromonas* and *Enterobacteria* as bacteria of public health concern, these and more pathogens were also reported in the current study.

4. Conclusion

This study confirms the presence of potential pathogenic and zoonotic fish bacteria in apparently healthy fish farms and aquatic ecosystems that can cause fish diseases and diseases to human beings.

Isolation of some bacteria in the kidney shows that such bacteria defeated the fish body defense mechanism and could be pathogenic and cause infection to the fish. Therefore, bacterial infection in *O. Jipe* maybe a reason for its extinction. This study recommends for proper biosecurity measures to be instituted to reduce potential bacteria and zoonotic pathogens.

5. Acknowledgements

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6.Statement of Competing Interests

The authors declare that they have no competing interests that could have influenced the conduct of this study or the interpretation of its results.

7. References

1. FAO. Report of the Intersessional Meeting of the Aquaculture and Fishery Subject Groups of the Coordinating Working Party on Fishery Statistics, London, 28–30 June 2023. In: FAO Fisheries and Aquaculture Report. Rome, Italy: FAO; c2024. Report No.: 1435. doi: 10.4060/cc9557en.
2. FAO. The State of World Fisheries and Aquaculture 2022. Rome: FAO; c2022. doi: 10.4060/cc0461en.
3. Obiero K, Cai J, Abila R, Ajayi O. Kenya: High Aquaculture Growth Needed to Improve Food Security and Nutrition. FAO; c2019.
4. Munguti J, Ogello E, Jeong-Dae H. An Overview of Kenyan Aquaculture: Current Status, Challenges, and Opportunities for Future Development. Fisheries and Aquatic Sciences. 2014;17:1-11. doi: 10.5657/FAS.2014.0001.
5. Ministry of Fisheries and Livestock Development. Project to Promote Fish Production in Taita Taveta Unveiled – Kenya News Agency. Ministry of Fisheries and Livestock Development; c2023. [Online]. Available: https://www.kenyanews.go.ke/project-to-promote-fish-production-in-taita-taveta-unveiled/ (accessed Jun. 15, 2024).
6. Opiyo MA, Marijani E, Muendo P, Odede R, Leschen W, Charo-Karisa H. A Review of Aquaculture Production and Health Management Practices of Farmed Fish in Kenya. International Journal of Veterinary Science and Medicine. 2018;6(2):141-148. doi: 10.1016/j.ijvsm.2018.07.001.
7. Shechonge A, Kihedu J, Ngatunga B, Bradbeer S, Day J. Widespread Colonisation of Tanzanian Catchments by Introduced *Oreochromis* Tilapia Fishes: The Legacy from Decades of Deliberate Introduction. Hydrobiologia. 2019;832(1):235-253. doi: 10.1007/s10750-018-3597-9.
8. Makilla D, Bett DK, Ngeje GK. A Comparative Study of Cage and Earthen Pond Culture of *Oreochromis Jipe* in Lake Jipe, Taita/Taveta District, Kenya; c2019.
9. Bradbeer SJ. Limited Hybridization Between Introduced and Critically Endangered Indigenous Tilapia Fishes in Northern Tanzani; c2019.
10. Omweno JO, Omondi R, Ondemo FM. Impacts of Introduced Species on the Fishery Potential, Ecology and Native Tilapia Populations of Lakes Victoria and Jipe, Kenya. In Review. Preprint; c2023. doi: 10.21203/rs.3.rs-2500229/v1.
11. Orina P, Matuma M, Akwany L, Kyule D, Okech J, Munguti J, Chepkerui M. An Overview of the Current Status of Lake Jipe and Its Biodiversity Dilemma. Lakes and Reservoirs. 2024, 29(1). doi: 10.1111/lre.12450.
12. Ndetei R. The Role of Wetlands in Lake Ecological Functions and Sustainable Livelihoods in Lake Environment: A Case Study on Cross Border Lake Jipe - Kenya/Tanzania.
13. Subasinghe R. Disease Control in Aquaculture and the Responsible Use of Veterinary Drugs and Vaccines: The Issues, Prospects, and Challenges. FAO; c2009.
14. World Health Organization. Regional Office for South-East Asia. Third Progress Analysis of Implementation of Antimicrobial Resistance National Action Plans in the WHO South-East Asia Region. New Delhi: World Health Organization; c2022. [Online]. Available: https://apps.who.int/iris/handle/10665/361822 (accessed Sep. 20, 2022).
15. International Office of Epizootics. Aquatic Animal Health Code; c2019.
16. Walakira J, Gichobi J, Balirwa J, Kimera R, Kabahenda M. Common Fish Diseases and Parasites Affecting Wild

and Farmed Tilapia and Catfish in Central and Western Uganda; c2014.
17. Legario FS, Choresca CH Jr, Turnbull JF, Crumlish M. Isolation and Molecular Characterization of *Streptococcal* Species Recovered from Clinical Infections in Farmed Nile Tilapia (*Oreochromis niloticus*) in the Philippines. Journal of Fish Diseases. 2020;43(11):1431-1442. doi: 10.1111/jfd.13247.
18. Akbar A, Ijaz M, Ehsan N, Kiran S. Control and Preventive Measures to Tackle Zoonotic Diseases from the Fish; c2023. doi: 10.47278/book.zoon/2023.018.
19. Wekesa C, Kirui BK, Maranga EK, Muturi GM. The Fate of Taita Hills Forest Fragments: Evaluation of Forest Cover Change Between 1973 and 2016 Using Landsat Imagery. Open Journal of Forestry. 2020;10(1):22-38. doi: 10.4236/ojf.2020.101003.
20. Bartlett JE, Kotrlik JW, Higgins CC. Organizational Research: Determining Appropriate Sample Size in Survey Research; c2001.
21. Richmond M, Mohammed A, Mwaitega S. Fish and Invertebrate Life Histories and Important Fisheries of the Pangani River Basin, Tanzania; c2008. doi: 10.13140/RG.2.2.18798.97601.
22. Roberts R. Fish Pathology. 4th ed. Hoboken, NJ: Wiley-Blackwell; c2012. doi: 10.1002/9781118222942.ch1.
23. Wanja DW, Mbuthia PG, Waruiru RM, Bebora LC, Ngowi HA, Nyaga PN. Antibiotic and Disinfectant Susceptibility Patterns of Bacteria Isolated from Farmed Fish in Kirinyaga County, Kenya. International Journal of Microbiology. 2020;2020:8897338. doi: 10.1155/2020/8897338.
24. Dissasa G, Lemma B, Mamo H. Isolation and Identification of Major Bacteria from Three Ethiopian Rift Valley Lakes Live and Processed Fish, and Water Samples: Implications in Sanitary System of Fish Products. BMC Veterinary Research. 2022;18(1):439. doi: 10.1186/s12917-022-03508-w.
25. Sanders ER. Aseptic Laboratory Techniques: Plating Methods. Journal of Visualized Experiments. 2012 May;(63):3064. doi: 10.3791/3064.
26. Austin B, Austin DA. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. 6th ed. Cham: Springer International Publishing; c2016. doi: 10.1007/978-3-319-32674-0.
27. Krieg NR, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman WB, editors. Bergey's Manual® of Systematic Bacteriology: Volume Four The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. New York, NY: Springer; c2010. doi: 10.1007/978-0-387-68572-4.
28. Mbuthia PG, Bebora LC, Nguta JM. Occurrence of Potential Pathogenic and Zoonotic Bacteria in Farmed Fish in Machakos and Nyandarua Counties, Kenya. International Journal of Fisheries and Aquatic Studies. 2022;10(6):112-118. doi: 10.22271/fish.2022.v10.i6b.2755.
29. Wanja DW, Mbuthia PG, Waruiru RM, Mwadime JM, Bebora LC, Nyaga PN, Ngowi HA. Bacterial Pathogens Isolated from Farmed Fish and Source Pond Water in Kirinyaga County, Kenya; c2020.
30. Pech ZG, Chavez MR, Reynoso LF. Pathogenic Bacteria in *Oreochromis niloticus* Var. Stirling Tilapia Culture. Fish and Aquaculture Journal. 2017, 8(2). doi: 10.4172/2150-3508.1000197.
31. Wanja DW, Mbuthia PG, Waruiru RM, Bebora LC, Ngowi HA. Natural Concurrent Infections with Black Spot Disease and Multiple Bacteriosis in Farmed Nile Tilapia in Central Kenya. Veterinary Medicine International. 2020;2020:8821324. doi: 10.1155/2020/8821324.
32. Dominguez MD, Mendiola NR, Roso GR, Quereda C. *Staphylococcus lentus* Peritonitis: A Case Report. Peritoneal Dialysis International. 2014 Jun;34(4):469-470. doi: 10.3747/pdi.2012.00303.
33. de Bruyn A, Jacobs K. Isolation of Potential Probiotic and Carotenoid Producing Bacteria and Their Application in Aquaculture; c2013.
34. Rajme-Manzur H, Velázquez-Valencia HD, Martínez-Porchas M, Vargas-Albores F, Coronado-Molina D, Hernández-Oñate M, Vázquez-Ramírez F, Santacruz A. *Staphylococcus haemolyticus* and *Providencia vermicola* Infections Occurring in Farmed Tilapia: Two Potentially Emerging Pathogens. Animals. 2023;13(23):3715. doi: 10.3390/ani13233715.
35. Silago V, Nyambura M, Mtebe M, Komba E, Salim M, Mgaya FX, Mirambo MM, Nyawale HA, Mshana SE, Matee MI. Multidrug-Resistant Uropathogens Causing Community Acquired Urinary Tract Infections Among Patients Attending Health Facilities in Mwanza and Dar es Salaam, Tanzania. Antibiotics. 2022 Nov;11(12):1718. doi: 10.3390/antibiotics11121718.
36. Arafa A. Survey on the most common bacterial pathogens of the Nile tilapia fries in Kafr El Sheikh Governorate, Egypt. Egypt J of Aquatic Biol and Fish. 2021 Mar;25(2):385-402. doi: 10.21608/ejabf.2021.164591.
37. Zulfa RM, Laith A, Kismiyati. Isolation and Identification *Bacillus* Bacteria in Tilapia (*Oreochromis niloticus*) Using the Vitek-2 Compact. IOP Conference Series: Earth and Environmental Science. 2021 Mar;718:012086. doi: 10.1088/1755-1315/718/1/012086.
38. Bernard CWK, Elias M, Muturi M. The Fate of Taita Hills Forest Fragments: Evaluation of Forest Cover Change between 1973 and 2016 Using Landsat Imagery. Open Journal of Forestry. 2020;10:22-38. doi: 10.4236/ojf.2020.101003.
39. Yusoff AZ, Nurul Amin, Nur Jasmin Y, Puvanasundram P, Murni K. Effects of Probiotics on Growth, Survival, Water Quality and Disease Resistance of Red Hybrid Tilapia (*Oreochromis* spp.) Fingerlings in a Biofloc System. Animals. 2021 Dec;11(12):3514. doi: 10.3390/ani11123514.
40. Ziarati M, Zorriehzahra MJ, Hassantabar F, Mehrabi Z, Dhawan M, Sharun K, Emran TB, Dhama K, Chaicumpa W, Shamsi S. Zoonotic diseases of fish and their prevention and control. The Veterinary Quarterly. 2022;42(1):95-118. doi: 10.1080/01652176.2022.2080298.
41. Basri R, Salleh A, Yasin S, Saad MZ, Rahaman Y, Barkham T, Amal M. Co-Infections of Tilapia Lake Virus, *Aeromonas hydrophila* and *Streptococcus agalactiae* in Farmed Red Hybrid Tilapia. Animals. 2020 Nov;10(11):2141. doi: 10.3390/ani10112141.
42. Rajan AA, Sugumar V, Vasantha P, Raja G. Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development.

Vaccines. 2023 Feb;11(2):470. doi: 10.3390/vaccines11020470.
43. Gawad HY, Abdel EA, Asely AME, Elabd H, Matter AF, Abbass AA. Insight into summer mortalities syndrome in farmed Nile tilapia (*Oreochromis niloticus*) associated with bacterial infections; c2020.
44. Basri NR, Salleh A, Yasin M, Saad MZ, Rahaman NY, Barkham T, Amal MN. Co-Infections of Tilapia Lake Virus, *Aeromonas hydrophila* and *Streptococcus agalactiae* in Farmed Red Hybrid Tilapia. Animals. 2020 Nov;10(11):2141. doi: 10.3390/ani10112141.
45. Bi B, Yuan Y, Jia D, Jiang W, Yan H, Yuan G, Gao Y. Identification and Pathogenicity of Emerging Fish Pathogen *Acinetobacter johnsonii* from a Disease Outbreak in Rainbow Trout (*Oncorhynchus mykiss*). Aquaculture Research. 2023 Feb;2023:1-13. doi: 10.1155/2023/1995494.
46. Cao S, Yi G, Geng Z, Deng L, Gan W, Wang K, Ou Y, Chen D, Huang X, Zuo Z, He M, Lai W. *Acinetobacter lwoffii*, an emerging pathogen for fish in *Schizothorax* genus in China. Transboundary and Emerging Diseases. 2018;65(6):1816-1822. doi: 10.1111/tbed.12957.
47. Mumbo MT, Nyaboya EN, Kinyua JK, Mathenge SGK, Rotich H, Muriira G, Njiraini B, Njiru JM. Antimicrobial resistance profiles of *Salmonella* spp. and *Escherichia coli* isolated from fresh Nile tilapia (*Oreochromis niloticus*) fish marketed for human consumption. BMC Microbiology. 2023;23:306. doi: 10.1186/s12866-023-03049-8.
48. Bedane TDM, Abunna F, Fufa W, Hila W, Woldemariyan FT, Tekle M, Shimelis E, Gutema FD. Occurrence, molecular characterization, and antimicrobial susceptibility of sorbitol non-fermenting *Escherichia coli* in lake water, fish and humans in central Oromia, Ethiopia. Scientific Reports. 2024 May;14(1):12461. doi: 10.1038/s41598-024-61810-z.
49. Stark J. First case of non-traumatic community-acquired *Pseudomonas fulva* infection. Indian Journal of Medical Microbiology. 2022;40(2):317-318. doi: 10.1016/j.ijmmb.2021.12.010.
50. Rahman AN, Mahboub HH, Elshopakey GE, Darwish IM, Gharib SA, Shaalan M, Fahmy EM, Abdel HM, Ismail SH, Elsheshtawy HM. *Pseudomonas putida* infection induces immune-antioxidant, hepato-renal, ethological, and histopathological/immunohistochemical disruptions in *Oreochromis niloticus*: the palliative role of titanium dioxide nanogel. BMC Veterinary Research. 2024;20:127. doi: 10.1186/s12917-024-03972-6.
51. An S, Kj S. Endo Microbial Fauna of Tilapia spp. (*Oreochromis niloticus*) found in a Flowing Canal at Eden Garden and Park Utako, Abuja. Journal of Fisheries and Livestock Production. 2017;5(1):doi: 10.4172/2332-2608.1000214.
52. Li G, Du X, Zhou D, Li C, Zheng Q, Cheng Z, Hu L, Zeng L, Meng W, Guo D, Sun L. Emergence of pathogenic and multiple-antibiotic-resistant *Macrococcus caseolyticus* in commercial broiler chickens. Transboundary and Emerging Diseases. 2018 Dec;65(6):1605-1614. doi: 10.1111/tbed.12912.
53. Cui Z, Hu L, Zeng L, Meng W, Guo D, Sun L. Isolation and characterization of *Priestia megaterium* KD7 for the biological control of pear fire blight. Frontiers in Microbiology. 2023 Mar;14:1099664. doi: 10.3389/fmicb.2023.1099664.
54. Zayet S, Lang P, Garnier A, Pierron J, Plantin J, Toko L, Royer PY, Villemain M, Klofenstein R, Gendrin V. *Leclercia adecarboxylata* as Emerging Pathogen in Human Infections: Clinical Features and Antimicrobial Susceptibility Testing. Pathogens. 2021 Oct;10(11):1399. doi: 10.3390/pathogens10111399.
55. Nugroho R, Saptiani G, SARINAH R, Agriandini M, Mawardi M. Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia. Biodiversitas. 2018 Mar;19:480-488. doi: 10.13057/biodiv/d190215.
56. Aly S, Nouh W, Salem-Bekhet M. Bacteriological and Histopathological Studies on *Enterobacteriaceae* in Nile Tilapia *Oreochromis Niloticus*. Journal of Pharmaceutical and Biomedical Sciences. 2012 Jan;2:94-104.
57. Rawash R, Saad S, Hassanin F, Hassan M, Maarouf A. Incidence of *Enterobacteriaceae* in some freshwater fishes. Benha Veterinary Medical Journal. 2019 Sep;37(1):64-68. doi: 10.21608/bvmj.2019.15658.1070.
58. Matabang GM, Miller D, Aggarwal R, Lafortuna A. First case report on *Empedobacter falsenii* bacteremia. IDCases. 2023 Jun;33. doi: 10.1016/j.idcr.2023.e01814.
59. Hang'ombe PB, Evensen Ø, Mutoloki S. *Lactococcus garvieae* isolated from Lake Kariba (Zambia) has low invasive potential in Nile tilapia (*Oreochromis niloticus*). Journal of Fish Diseases. 2021 Feb;44:doi: 10.1111/jfd.13339.
60. Sundar RS, Lekshmi N, Arathi D, Sood N, Kumar PK, Ramacha S, Lal K. Co-infection of *Lactococcus garvieae* and Tilapia lake virus (TiLV) in Nile tilapia *Oreochromis niloticus* cultured in India. Diseases of Aquatic Organisms. 2021 Dec;147:127-140. doi: 10.3354/dao03638.
61. Abraham TJ, Paul P, Adikesavalu H, Patra A, Banerjee S. *Stenotrophomonas maltophilia* as an opportunistic pathogen in cultured African catfish *Clarias gariepinus* (Burchell, 1822). Aquaculture. 2016 Jan;450:168-172. doi: 10.1016/j.aquaculture.2015.07.015.
62. Chitambo B, Munyeme M, Hang'ombe B. Identifying Bacteria with Public Health Significance from Farmed Nile Tilapia (*Oreochromis niloticus*), Zambia. International Journal of Microbiology. 2023 Jun;2023:6650378. doi: 10.1155/2023/6650378.
63. Roh H, Kannimuthu D. Assessments of epidemic spread in aquaculture: comparing different scenarios of infectious bacteria incursion through spatiotemporal hybrid modeling. Frontiers in Veterinary Science. 2023 Sep;10:1205506. doi: 10.3389/fvets.2023.1205506.

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