Ecohydrology & Hydrobiology 13 (2013) 261–266

Contents lists available at ScienceDirect

Ecohydrology & Hydrobiology

journal homepage: www.elsevier.com/locate/ecohyd

Original Research Article

Microbial assessment of selected earthen fish ponds in western Kenya

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ARTICLE INFO

Article history:
Received 18 August 2013
Accepted 2 October 2013
Available online 22 October 2013

Keywords:
Enteric
Earth fish ponds
Oreochromis niloticus

ABSTRACT

Water-borne infections are among the most recent emerging and re-emerging infectious diseases throughout the world. WHO estimates that 80% of all illnesses in the world are caused by water-borne disease pathogens that thrive due to inadequate sanitation and polluted water. This study determined the presence of enteric microbes in medium earthen fish farm ponds and waters in the region. Fifty-seven *Oreochromis niloticus* L. and 36 water samples were collected over three months from 12 ponds within Maseno and Luanda Division. Sixty-six enteric microbes were found – *Vibrio hollisae* (18.2%), *Proteus vulgaris* (12.1%), *Yersinia* spp. (7.6%), *Salmonella typhi* (7.6), *Aeromonas hydrophilia* (7.6%), *Edwardsiella tarda* (6.1%), and *Escherichia coli* (6.1%) were the most isolated Enterobacteriaceae from water. Fifty enteric microbes were collected from fish intestines; *Citrobacter freundi* (62%) and *Proteus* spp. were the most common. Thirty-nine bacteria were isolated from the macerated fish flesh with *Citrobacter* spp., *Proteus* spp. and *Pseudomonas* being the most common. Water temperature, salinity dissolved oxygen and pH were within the expected range. Earthen fish ponds harbor enteric microbes that could be pathogenic to humans, although they had low faecal bacterial indicators signifying minimal contamination from human waste.

1. Introduction

Water-related diseases are typically placed in four classes: waterborne, water-washed, water-based, and water-related insect vectors (Rose et al., 2001; Gleick, 2002). Surface-water contamination has been associated with excessive rainfall. Utilization of rainfall run-off waters has been associated with faecal contamination in the vicinity of farms, because of percolating waters with human sewage, farm effluents and wild animal contamination increasing prevalence of pathogens (*Salmonella*, *Shigella*, pathogenic *Escherichia coli*, enteric viruses, hepatitis viruses, parasites) (Harvell et al., 2002; Hunter, 2003).

The Nile tilapia, *Oreochromis niloticus* L. (Cichlidae), is the world's major cultured species of tilapia, with 603,034 tons produced in 1996 (Medema et al., 2003). In recent years, aquaculture has become a very fast growing sector

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http://dx.doi.org/10.1016/j.ecohyd.2013.10.005

in animal production. However, the number of outbreaks of bacterial diseases in cultured fish has also increased. The existence of a semi-permanent store of faecal bacteria in catchment soils combined with hydrological transport mechanisms capable of moving bacteria from land to stream channels have been suggested (Medema et al., 2003). Food safety issues associated with aquaculture products vary according to the method of production, management practices and environmental conditions (Reilly and Käferstein, 1999). Being unaware of micro-organisms present in aquaculture products can affect human health as evidenced by the transmission of streptococcal infections from tilapia to humans resulting in several cases of meningitis in Canada (Prevention C.F.D.C.A., 1996). Thus, the main mechanisms for regulation of bacterial populations in the aquatic environment need to be studied, in order to enhance food safety, monitoring of harvest areas for faecal pollution and microbiological analysis products. Microbiological research has generally focused upon detecting the harmful intestinal bacteria, and to the methods to control them.

Global warming is causing the emergence, resurgence and spread of infectious diseases, killing millions of people annually (Anthes et al., 2007). Bacterial diseases are mainly caused by opportunistic (facultative) bacterial pathogens, which can reside in the environment or on/in apparently normal fish (latent carriers) (Wedemeyer, 1996). Interactions between fish and microorganisms that are present in the aquatic environment are a potentially serious source of mortality but under standard conditions of hygiene are often harmless. Infections are often precipitated by some stress (e.g. overcrowding, low dissolved oxygen, high ammonia, high temperature, or various pollutants) that upsets the natural defenses (Noga, 1996) and allow bacteria to colonize, penetrate, and invade host tissues. Bacteria are an additional pollutant released from aquaculture systems (Austin and Austin, 1989). Bacteria population in aquaculture systems as a result of waste can lead to infections in wildlife as well as exposure of humans to possible pathogens thus need to be monitored.

Aquaculture is the fastest growing agricultural industry today and the systems generally employ intensive culture methods involving dense populations of fish. These culture methods can produce large quantities of waste often released into natural bodies of water. The refuse consists largely of unused feed and excretory waste. This dense waste leads to algae blooms causing a decrease in the growth of natural flora and fauna. Some algae blooms produce toxins that can further decrease the natural fish populations and lead to disease in humans (Austin, 1999).

Closed aquaculture systems have been proposed for production of fish in areas of low water availability or unfavorable water conditions for specific fish species, as well as to produce fish closer to their markets (Stickney, 1993). This is why farmers in western region settled to use rain runoff, and stream as water source for their fish ponds after the advice from the Kenya Lake Basin Authority program to curb poverty and improve on food insecurity.

Therefore, it was imperative to perform microbial assessment of selected water bodies in western Kenya in order to ascertain their suitability for fish farming.

2. Materials and methods

Fifty seven Nile tilapia, *O. niloticus* L. (Cichlidae) and 36 water samples were collected from 12 earthen fish pond in Kisumu Rural and Emuhaya Constituencies, during end of dry and early long rainy season (March; April–May 2009), in Maseno, western Kenya at 0°0′0″ South, 34°36′0″ East. Emuhaya constituency has two administrative divisions: Emuhaya and Luanda 0°0′0″ South, 34°36′0″ East (www.maplandia.com/kenya/western/maseno). It has an average altitude of 1503 m a.s.l. (http://www.fallingrain.-com/world/KE/7/Maseno.html, accessed on 28.09.13), temperature range from 18 to 25 °C. This area receives heavy rainfall (mean annual precipitation 1250 mm) throughout the year with a high humidity. It has a population of 65,304, of which 2199 are classified as urban (KNBS, 1999). The fish farms were owned and managed by six families (six ponds) except in Emuhaya, which were owned and managed by Community Self-Help groups.

Three ponds in the study area were small while nine were medium, where for the purpose of this research, a pond was small if it had less than 5000 fish, medium between 5000 and 10,000 fish and large pond more than 10,000 fish. Three ponds received water from the groundwater and nine received water from diverted streams. The commonly stocked fish species were the Nile tilapia (*O. niloticus* L.). However though not in the interest of this study, the African catfish, *Clarias gariepinus* were found to be mixed with the tilapia in order to reduce tilapia population by predations.

2.1. Water sample collection and microbial analysis

The surface water samples were randomly collected in triplicates using 500 ml plastic bottles after being washed with sodium hypochlorite, rinsed thoroughly with sterilized distilled water and 70% ethyl alcohol. The water samples were then transported to the microbiology laboratory at Maseno University within 6 h of collection where they were analyzed for the common Enterobacteriacea.

Fourteen milliliters of water from each sample were filtered using sterile filter paper to remove all the solid debris. The filtrate was then centrifuged at 2400 rpm for 20 min to concentrate the microorganisms. A pellet was obtained (1×10^6) colony forming units (CFU) according to MacFaland technique using a pipette and inoculated on to nutrient broth medium and incubated at 37 °C for 24 h, so as to enhance the growth of all bacteria present in the water. Inocula from nutrient broth were then subcultured in MacConkey, Deoxycholate Citrate Agar, Xylose Lysine Deoxycholate Agar, SC, Salmonella Shigela Agar, EMB, TCBS, Brain-Heart Infusion Agar (BHIA), Tryptic Soy Agar (TSA), Kligler Iron Agar and Simmon Citrate Agar.

A total of twelve fish farms were studied, where three ponds were randomly selected from each farm if there were more than three fish ponds. If there were three or less ponds on the farm, all the ponds were used for the study. Five fish (*O. niloticus* (L.)) were collected randomly from the selected ponds in each farm using a fishing net and they were packed individually in sterile plastic bags and

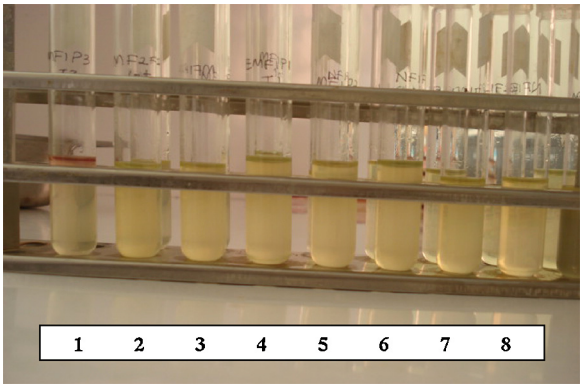

Fig. 1. Indole test: peptone water after addition of Kovac's reagent. Tube 1 shows a positive test while tubes 2–8 show negative tests.

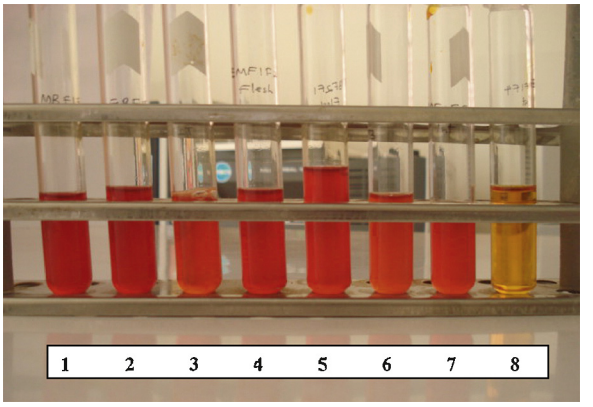

Fig. 2. Methyl red test in MRVP media. Tubes 1–7 present positive reaction for the mixed acid pathway and tube 8 present a negative test.

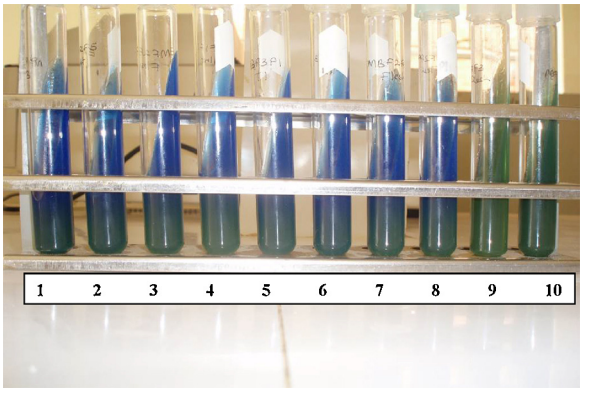

Fig. 3. Citrate utilization test on Simmon's citrate agar slant. Tubes 1–8 present positive test for citrate utilization, while tubes 9 and 10 show a negative test.

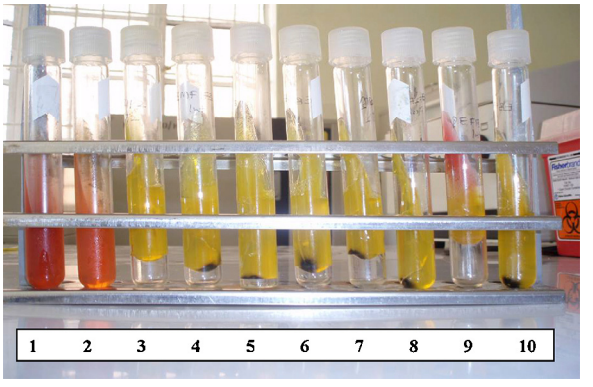

Fig. 4. Triple sugar iron (TSI) slants showing negative tests in tubes 1 and 2; hydrogen sulphide in tubes 4–8 and 10. Tubes 3–10 have acidic butt and gas production, and acid slant except tube 9.

transported in a cool box to the laboratory at Maseno University for analysis within 2 h of capture. 57 Nile tilapia (*O. niloticus*) were collected for the study in this case.

2.2. Analysis of fish samples

In the laboratory the fish were disinfected by dipping in 70% ethyl alcohol for 2 min then rinsed 3 times with sterilized distilled water according to Newaj-Fyuzul et al. (2006). The flesh and the intestines were separated from the fish and macerated using a disinfected mortar and pestle, and then mixed with sterilized PBS to a concentration of 10% (w/v). 5 ml of the slurry was used to inoculate SSA, BHIA, TSA and tubes of selenite faecal (SF) broth (HIMedia Laboratories Pvt. Ltd., Mumbai, India) which were incubated for 18–24 h at 37 °C. Possible *Salmonella* colonies were streaked on brilliant green agar (BGA) (Fluka, Sigma–Aldrich Chemie GmbH, Switzerland) and Xylose Lysine Deoxycholate (XLD) agar (HIMedia Laboratories Pvt. Ltd., Mumbai, India). The isolates were confirmed to be *Salmonella* by a series of biochemical tests such that they were negative for indole production when Kovac's reagent was added to 24-h cultures in peptone water (Fig. 1).

All the isolates were subjected to the Methyl Red Voges Proskauer test where indole production was checked by inoculating the isolates into tubes of peptone water (HIMedia Laboratories Pvt. Ltd., Mumbai, India) then incubated at 37 °C for 24 h. Five drops of Kovac's reagent (HIMedia Laboratories Pvt. Ltd., Mumbai, India) were added to each tube and observation recorded (Fig. 1). The MRVP broth was divided into two tubes. 5 drops of methyl red (MR) indicator were added to the first tube to test for mixed acid metabolic pathway. Five drops of α-naphthol solution were added to the second tube, shaken for 1 min then 5 drops of 40% potassium hydroxide solution were added to the same tube to test for the Voges Proskauer (VP) metabolic pathway. Observation were made after 1 min and recorded (Fig. 2). The isolates' citrate utilization was established by inoculating them onto Simmon's citrate (SC) agar (HIMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37 °C for 48 h then observations made (Fig. 3). The carbohydrate fermentation pattern, hydrogen sulphide and gas production was established by inoculation into tubes of Triple sugar iron (TSI) agar (Fluka, Sigma–Aldrich Chemie GmbH, Switzerland) then incubated at 37 °C for 24 h. The isolates were stored in Tryptic soy broth (TSB) at 4 °C for future reference (Fig. 4).

Table 1
Predominant bacteria isolated from water, fish intestine and fish slurry from selected fish ponds in Emuhaya constituency. Similar strains have been summed up to provide the strain profile in the respective isolation environment.

Microorganism	No. of isolates	Total isolates	% isolates ($n = 66$)
Shigella sonnei	6		
Shigella dysenteriae	1	**7**	10.6
Escherichia coli	4	4	6.06
Salmonella typhi	5	5	7.57
Klebsiella pneumoniae	1		
Klebsielle oxytoca	2	**3**	4.54
Enterobacter aerogenes	3	3	4.54
Proteus vulgaris	8	8	12.12
Pseudomonas aeruginosa	2	2	3.03
Yersinia spp.	5	5	7.57
Edwardsiella tarda	4	4	6.06
Citrobacter freundi	2	2	3.03
Acinetobacter spp.	1	1	1.51
Vibrio cholera	5		
Vibrio mimicus	3		
Vibrio parahaemolyticus	3		
Vibrio hollisae	1	**12**	18.18
Pleisiomonas shigelloides	2	2	3.03
Aeromonas hydrophilia	5	5	7.57
Others (non-identifiable)	3	3	4.54
Total	66	66	

The bold values signify the total isolates for the respective microbial genus.

Table 2
Microbial profile isolated from flesh and intestine of *Oreochromis niloticus* L. from selected fish ponds in Emuhaya constituency. Similar strains have been summed up to provide the strain profile in the respective isolation environment.

Microorganism	No. of isolates	Total isolates	% isolates ($n = 50$)
Intestines			
Citrobacter spp.	1	1	2
Citrobacter freundi	31	31	62
Enterobacter aerogenes	2	2	4
Proteus mirabilis	9		
Proteus vulgaris	3	**12**	**24**
Providencia??	1	1	2
Pseudomonas	1	1	2
Shigella spp.	1	1	2
Yersinia spp.	1	1	2
Total		50	100
Flesh (n = 39)			
Proteus mirabilis	5		
Proteus vulgaris	1	**6**	**15.38**
Escherichia coli	1	1	2.56
Pseudomonas	2	2	5.12
Total		39	100

The bold values signify the total isolates for the respective microbial genus.
??, The respective isolates could not be determined to the species level due to their doubtful biochemical reactions in various media.

3. Results

3.1. Composition of enteric bacteria in the respective fish pond

The major Enterobacteriacea isolated in fish ponds water were *Vibrio hollisae* 18% ($n = 12/66$), 12% ($n = 8/66$) *Proteus vulgaris*, 10.6% ($n = 7/66$) *Shigella dysenteriae*, 7.6% ($n = 5/66$) *Salmonella typhi*, 7.6% ($n = 5/66$) *Yersinia* spp., 7.6% ($n = 5/66$) *Aeromonas hydrophilia*, 6.1% ($n = 4/66$) *Edwardsiella tarda*, 6.1% ($n = 4/66$) *E. coli*, 4.5% ($n = 3/66$) *Klebsiella* spp., 4.5% ($n = 3/66$) *Enterobacter aerogenes*, 3% ($n = 2/66$) *Pseudomonas aeruginosa*, 3% ($n = 2/66$) *Citrobacter freundi*, 3% ($n = 2/66$) *Pleisiomonas shigelloides* and 4.5% ($n = 3/66$) others non-identifiable species respectively (Table 1).

The Enterobacteriacea isolated from fish intestine contained a more limited bacterial flora – 62% ($n = 31/50$) *C. freundi*, 18% ($n = 9/50$) *Proteus mirabilis*, 4% ($n = 2/50$) *E. aerogenes*, 6% ($n = 3/50$) *P. vulgaris*, 2% ($n = 1/50$) *Citrobacter* spp., 2% ($n = 1/50$) *Providencea*, *Pseudomonas*, *Shigella* spp., and *Yersinia* spp., respectively (Table 2).

In the flesh were 12.8% ($n = 5/39$) *P. mirabilis*, 2.6% ($n = 1/39$) *P. vulgaris*, 2.6% ($n = 1/39$) *E. coli*, and 5.1% ($n = 2/39$) *Pseudomonas*. The mean physicochemical parameters of the ponds were found to be within the expected ranges except from a few that had values that would favor the growth and survival of the pathogenic microbes (Table 3).

4. Discussion

Pathogenic vibrio strains responsible for human and veterinary infections has so far been classified in 35 most recognized species with 12 being considered to human pathogens (Farmer and Hickman-Brenner, 1992). Among those strains classified as other strains of vibrio is *V. hollisae* (*Grimontia hollisae*) that is considered responsible for cases for minor pathogenicity (Yamane et al., 2004). In our study, we isolated a higher frequency of *V. hollisae* (*G. hollisae*) in fish slurry from the fish pond an implication of cross contamination from environment (Adebayo-Tayo et al., 2012; Elhadi, 2013). This isolation would point at gastrointestinal infections by this bacteria leading to diarrhea in the region. Though no clinical sampling was done, it is documented that *G. hollisae* (*V. hollisae*), and *V. mimicus* can cause diarrhea or infections of the gastrointestinal tract in humans (Farmer and Hickam-Brenner, 2003; Farmer et al., 2003; Adeleye et al., 2010). This is an indication that if there is no proper cooking of the fish as well as maintenance of good personal hygiene to avoid coming into contact with the fish and already contaminated water, there could be human health hazard associated with intestinal disease or extra intestinal disease, especially wound infections, due to exposure to

Table 3
Mean measured and expected values of physicochemical water parameters of the ponds where respective fish samples were harvested.

Water physiological parameters	Mean observed measurements	Expected measurements
Dissolved oxygen (mg/L)	2.695	≥2.3
pH	5.208	6.5–9
Salinity (ppt)	0.263	0–2.5
Temperature (°C)	24.935	20–35

pH, preferential hydrogen; ppt, parts per thousand; °C, degree Celsius.

the aquatic environments and handling of fish. *V. parahaemolyticus* inhabits the marine and brackish water environments and it is therefore associated with fishes harvested from these environments (Anwar et al., 2010). However, the bacterium was isolated from fresh water fish and indication of low sodium levels though not studied (Farmer and Hickam-Brenner, 2003; Farmer et al., 2003). It is reported to these serotype exhibits increased adherence and cytotoxicity in tissue culture and this may contribute to the enhanced pathogenic potential of strains of this serotype. It is also inferred that the isolated *A. hydrophilia* in this fish could be a result of some injured fish in the pond due to stress generated by overcrowding of the ponds. Similar results were reported by Cabral (2010). The common routes of infection suggested for *Aeromonas* are the ingestion of contaminated water or food or contact of the organism with a break in the skin. Among the other bacteria isolated from the fish in the environment was *Shigella dysenterie*. It has been found that although numbers of cultivated cells are low in environmental samples (waters and fish) could display shigellosis in genetic elements. A phenomenon shared between the cholera and the shigellosis cycles in the environment. It remains to be elucidated if *Shigella* can also exist in environmental waters in a viable but non-culturable state, as vibrios (Faruque and Mekalanos, 2003). *Citrobacter* is reported to occur in environments such as water, sewage, soil and food (Frederiksen and Søgaard, 2003; Frederiksen, 2005) thus this created a possibility of the fish being contaminated. It is documented that the ability of faecal bacteria to survive in environmental waters increases as the temperature decreases, in all these senarios the average water temperature in all the ponds was found to be 24.9 °C. Additional factors that affect their survival in the environment include dissolved organic carbon concentration, sunlight intensity and the ability to enter the viable but non-culturable state (Medema et al., 2003). In this regard it was found that the average dissolved oxygen in the ponds was 2.695 which were slightly above the expected of 2.3. Their survival in soil (and concomitantly in groundwater) is reported to be enhanced by low temperatures, high soil humidity, neutral or alkaline soil pH and the presence of organic carbon (Medema et al., 2003). The recorded pH in this study was 5.2 which were lower than the expected 6.5. Based on the findings of this study it is reported that according to the available microbiological guidelines by Gilbert et al. (1996), the microbiological quality of the selected pond fish examined herein is unacceptable and pose a potential risk to public health since the fish are reported to harbor both opportunistic and pathogenic bacteria. These findings were similar to those by Mhango et al. (2010) and the presence of these coliforms in fish is an indication of pollution of their environment.

5. Conclusion

The waters feeding the fish ponds in Emuhaya constituency is contaminated with faecal coliforms that would eventually infect the fish while feeding or when there are injuries inflicted by other fish or by handling in the fish pond. This make the fish to be a conduit of pathogenic microbes to humans if they feed on food that is not properly cooked.

Conflict of interest

There is no conflict of interest.

Financial disclosure

The research study was funded by funds from Dr. David Onyango. The funds holistically took care of the research logistics from the beginning to the end. We acknowledge Maseno University for providing the logistics of the study. We also thank the administrative authorities of Emuhaya constituency and fish farmers for having consented to the study.

Acknowledgements

The team would like to thank Dr. David M. Onyango for providing research funds to run this study from his personal coffers. He also provided the road map to the realization of this study. We also want to thank Mr. James Ochieng Ochieng for being handy in collecting both fish and water samples from the respective pond sites and also in assisting in part of the sample analysis. We would like to thank Mr. Jeremiah Were for his lab work contribution in analyzing the samples together with Dr. Onyango, Ochieng and partly with Prof. Waindi. Professor Waindi gave the scholarly advice and also provided laboratory space and other reagents for the realization of this study. We want to acknowledge Department of Zoology, Maseno University for providing the laboratory space, reagents and materials to undertake this study. We also want to thank the fish farmers who gave us consent to use their fish farms (ponds) for this study. We are grateful to all.

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