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Full Length Research Paper

The Bacteriology and Physico-chemical Analysis of Freshwater Fish Ponds

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ABSTRACT

A survey of the bacteriological and physico-chemical analysis of three freshwater earthen fish ponds stocked with *Clarias gariepinus* was conducted using standard methods. The antibiotics susceptibility testing of the bacterial isolates were determined using the agar disc diffusion method. The total aerobic bacterial count ranged from 1.50 x 10^4 CFU/mL – 1.13 x 10^6 Cfu/mL. Twenty-five bacterial isolates belonging to thirteen genera were identified. The values of the physico-chemical parameters were within the acceptable limit. Potential pathogens of which 80 per cent exhibited multiple antibiotic resistance was observed. The study suggests the need for periodic water quality control of fish ponds in order to ensure fish safety as well as to prevent the transmission of potential pathogens with multi-drug resistance to humans.

Keywords: Fish pond, Physico-chemical, pathogens, antibiotics, resistance

INTRODUCTION

Aquaculture is a major fast growing sector of global food production and a source of animal protein in the world today. In Nigeria, the rearing of African catfish (*Clarias gariepinus*) is proving an immense role in the provision of food and its usefulness cannot be over-emphasised as the demand for fish is expanding rapidly throughout the world. However, fish farming at present has been observed not to be a likely replacement for deficiencies in annual catch rates from the sea because of the enormous differences in volume of production (Purdom, 1996) and chiefly as a result of the emergence of infectious diseases. Microorganisms contribute a significant fraction of importance in the aquatic ecosystem and they have been observed to be among the factors that can cause the emergence of infectious diseases in the practice of aquaculture (Noga, 2000; Ikpi and Offem, 2011).The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment (Boon and Huisman, 1996; Noga, 2000).

The distribution and size of fish populations are largely determined by the interaction of the fish with the immediate environment which directly impact on pond water quality and indirectly on the whole ecosystem. Moreso, the placing of fish in fish ponds may expose them to new pathogens. Great loss of fish has been attributed largely to bacterial infections (Sasson, 1990). The flesh of fish is usually infected with a wide range of microorganisms present in the water body (Pyakin and Krivoshein, 1986), hence the bacteria flora of the fish depicts the level of bacteria of the water environment. They become pathogenic when conditions such as temperature changes, dietary, hormonal stresses and other physico-chemical parameters are favourable for the development of pathogenesis (Wiloughby, 1976; Svobodova et al., 1993).

Fish is an important component of human foods and animal feeds. Hence the need for rapid development and proper management of fishery is becoming a necessity in view of the high demand for fish as a relatively cheap

source of animal protein. Water is the most important resource for aquaculture and can be a significant source for contamination. The conditions that fishes are cultured may be potentially stressful, causing existing infections to become more severe and precipitate disease outbreaks which may also compromise the fitness of such fish for human consumption. According to Ugwuba and Chukwuji (2010), the mortality of fish due to disease and water pollution constitutes problems to aquaculture development in Nigeria. This study aims at identifying the bacterial isolates, determine antibiotics susceptibility pattern and analyse some physico-chemical parameters of water from some fish ponds in Ile-Ife, Nigeria.

MATERIALS AND METHODS

Water samples collection

Water samples were collected from three earthen fish ponds stocked with the African catfish (*Clarias gariepinus*) sited at different locations in Ile-Ife during the months of October-December at 10-12 GMT time. 100mls of water were collected in sterile glass wares containers with stoppers. The water samples for microbial analysis were transported in ice-packs and processed between 2-3 h after collection.

Microbial Analysis

The microbial load of the water from the fish ponds was determined by performing a ten-fold serial dilution of the sample in test tubes containing sterile distilled water. The total viable bacterial count was determined using the pour plate technique cultured in duplicates. The plates were incubated at 35^{o}C for 48 h. The colonies were counted and expressed in colony forming unit per ml (cfu/ml) and values were estimated by means of duplicate determination. The isolated colonies were streaked on nutrient agar and pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests (Cheesbrough, 2006) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Antibiotics Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates was determined by the Kirby-Bauer agar disc diffusion technique (Bauer *et al*., 1966). The plating medium was Mueller-Hinton agar and antibiotics used contained Amoxicillin (25 µg), Ofloxacin (5µg), Streptomycin (10µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Gentamicin (10µg), pefloxacin (5µg), Cotrimoxazole (25µg), Ciprofloxacin (10 µg), Erythromycin (5µg), Augmentin (30µg), Nitrofurantoin (20µg) and Tetracycline (30µg) (Fondoz, Nigeria).

Multiple Antibiotics Resistance (MAR) Indexing of Isolates

The multidrug resistance of the bacterial isolates were analysed using the multiple antibiotic resistance (MAR) index. The multiple antibiotic resistance (MAR) index was defined as a/b where 'a' represent the number of antibiotics to which the isolate is resistant to and 'b' the number of antibiotic to which the isolate is exposed. The isolates with MAR index values higher than 0.2 were considered multiple resistant (Krumperman, 1983).

Physico-chemical Analysis of the fish pond water

The physico-chemical parameters of the fish ponds water were analysed within the holding time of each parameter, following standard methods.

Water temperature was measured *in-situ* using mercury-in-glass bulb thermometer with calibration range (-10 ^{0}C – 110 ^{0}C) between 10-12 GMT. Samples for the physico-chemical parameters were collected in a clean 2 litres plastic bottles (except samples for Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD)). Glass reagent bottles were used to collect samples for DO determination and the samples were fixed on the field immediately after collection with Winkler's reagent (Manganous sulphate and Alkaline Iodide) and the oxygen content determined by iodiometric titration with N/40 Sodium Thiosulphate solution (Golterman et al., 1978) while dark reagent bottles were used for BOD determination and were kept in a dark cupboard for 5 days for subsequent analysis. Sample pH was measured using a pH meter with a glass electrode (Jenway, 3020 model), while electrical conductivity was measured with conductivity meter (Jenway, 4071 model) at 25 ^{0}C.

The total solids (TS) as well as total dissolved solids (TDS) of the samples were determined gravimetrically after the samples were oven dried to constant weight at 105 ± 2 ^{0}C. Total suspended solids (TSS), was calculated as the difference between TS and TDS (USEPA, 1983). Total acidity, total alkalinity and chloride ions (Cl^{-}) contents were determined by titrimetric methods (Golterman et al., 1978) while sulphate (SO_4^{2-}), nitrate (NO_3^{-}) and phosphate (PO_4^{3-}) ions were determined by spectrophotometric methods (Ademoroti, 1996). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions were determined by complexio-metric titration method using Na_2EDTA while Sodium (Na^{+}) and Potassium (K^{+}) ions were determined by the atomic emission spectrophotometric method using a flame analyzer (Golterman et al., 1978). Total water hardness was determined as estimates of the total concentrations of the calcium (Ca^{2}+ and magnesium (Mg^{2}+) ions predetermined by complexio-metric titration

Table 1. Occurrence of the bacterial isolates in the fish ponds

Organisms	Number of isolates	Pond A	Pond B	Pond C
Aeromonas sp.	3	+	+	+
Pseudomonas sp.	3	+	+	+
Klebsiella sp.	3	+	+	+
Micrococcus sp.	2	+	-	+
Acinetobacter sp.	2	+	+	-
Bacillus sp.	2	+	+	-
Staphylococcus aureus	2	+	+	-
Proteus sp.	2	+	-	+
Escherichia coli	2	+	+	-
Enterobacter sp.	1	+	-	-
Moraxella sp.	1	+	-	-
Shigella sp.	1	+	-	-
Streptococcus faecalis	1	+	-	-

+ = cultured; - = not cultured

method using Na_2EDTA. Colour was determined colorimetrically using a colorimeter (Jenway, 6051 model) standardized with a set of potassium chloroplatinate-cobalt solutions as standards, while turbidity was determined using a turbidimeter with values expressed in nephelometric turbidity units (NTU) (Ademoroti, 1996). All the recommended quality control (QC) and quality assurance (QA) measures were taken for each determination.

RESULTS

The mean averages of the total viable bacterial count (cfu/ml) from the three ponds sampled were as follows: Pond A = 1.13×10^6, Pond B = 1.50×10^4 and Pond C = 1.14×10^5

Twenty five bacterial isolates belonging to thirteen genera were identified from the three fish ponds. The microorganisms and their occurrence pattern are as shown (Table1).

Of the 25 isolates tested for *in-vitro* antibiotic susceptibility, 20 (80%) of the isolates exhibited multiple antibiotic resistance (Table 2).

The value ranges for all the three ponds sampled showed a temperature range of 27-29^oC, pH 6.65- 7.80, Alkalinity 18-112 $mgCaCO_3l^{-1}$, total hardness 17.81-166.52 $mgCaCO_3l^{-1}$, Dissolved oxygen (DO) 0-4.8 mgl^{-1}, Biochemical oxygen demand (BOD) 2.64- 6.4 mgl^{-1}. The values for the other physico-chemical parameters are as shown (Table 3).

DISCUSSION

It has been observed that infectious disease is one of the most important constraints to efficient and sustainable aquaculture production, impacting on food security, socio-economic development, profitability and trade (Walker, 2004). The persistence of pathogens in the water environment also is considered as one of the crucial factors for infection transmission (Mlejnková and Sovová, 2012) in terms of acute outbreaks of disease. In this study, the microbial analyses of the water collected from the fish ponds revealed a high microbial load. The finding is in agreement with the report of Oni et al. (2013) in a study on associated microbial load of artificially cultured *C. gariepinus* fingerlings, in which high microbial load with low mortality of the fingerlings was recorded. This may be an indication that *C. gariepinus* is adequately suited to withstand high microbial load.

The study revealed the presence of *Escherichia coli, Aeromonas sp. Klebsiella sp., Staphylococcus aureus* and *Shigella spp* in the fish ponds water may pose a threat to the health of the fishes and consumers. Gram negative bacteria were the most predominant bacteria isolated and this is in agreement with findings reported by (Molokwu and Okpokwasili, 2002; Akinwale et al., 2007) in their study on the diversity of microflora of fish pond water. The recovery of *Escherichia coli* and *Streptococcus faecalis* in two of the fish ponds could suggest a possible faecal contamination of the fish ponds. The MAR index revealed that 80 per cent of the isolates showed multiple antibiotic resistance indicating that the pond water as well as the fish could be a reservoir for multidrug resistance. This may have adverse health burden on the consumers.

According to Meade (1989) physico-chemical parameters such as alkalinity, dissolved oxygen, total hardness pH and temperature are the most common water quality characteristics that will influence fish health and growth. The finding from the physico-chemical analysis revealed values that are within the range approved for warm water fish. The temperature range of 27-29^oC is within the target water quality range for growth (Department of Water and Forestry, 1996). The pH of the

Table 2. Multiple Antibiotics Resistance of the isolates

Number of antibiotics used (b)	Number of Resistant Isolates (a)	MAR Index a/b	Frequency of MAR (%)
	3	0.3	1
	4	0.4	3
10	5	0.5	2
	6	0.6	1
	7	0.7	10
	8	0.8	2
	9	0.9	1
			20 (80%)

Table 3. Physico-chemical water quality parameters

Parameter (unit)	Pond			Descriptive Statistics	
	A	B	C	Mean±S.E.	Range
Temperature (^oC)	29.0	27.0	28.0	28.0±0.58	27.0-29.0
Apparent colour (Pt-Co)	198.1	349.5	147.9	231.8±60.6	147.9-349.5
True colour (Pt-Co)	0.86	0.86	6.02	2.58±1.72	0.86-6.02
Turbidity (NTU)	15.84	21.18	42.54	26.52±8.16	15.84-42.54
TDS (mgl^{-1})	260	130	50	146.7±61.2	50-260
TSS (mgl^{-1})	80	100	180	120±30.6	80-180
TS(mgl^{-1})	340	230	230	266.7±36.7	230-340
pH	7.80	6.65	7.63	7.36±0.36	6.65-7.80
Conductivity (μScm^{-1})	431.0	213.0	87.1	243.7±100.5	87.1-431.0
Acidity ()	10	22	14	15.3±3.5	10-22
Alkalinity ($mgCaCO_3l^{-1}$)	74	18	26	93.3±17.5	18-74
Total Hardness ($mgCaCO_3l^{-1}$)	109.82	82.35	17.81	69.99±27.27	17.81-109.82
DO (mgl^{-1})	0.0	2.4	4.8	2.4±1.4	0.0-4.8
DO Saturation(%)	0.0	30.53	61.94	30.82±17.88	0.0-61.94
BOD (mgl^{-1})	4.4	5.6	6.4	5.5±0.6	4.4-6.4
Ca^{2+}(mgl^{-1})	37.58	30.20	6.19	24.66±9.48	6.19-37.58
Mg^{2+} (mgl^{-1})	3.87	1.67	0.57	2.04±0.97	0.57-3.87
Na^{+} (mgl^{-1})	38.84	24.84	18.84	27.51±5.93	18.84-38.84
K^{+} (mgl^{-1})	21.07	13.60	2.15	12.27±5.50	2.15-21.07
Cl^{-} (mgl^{-1})	19.08	8.58	2.35	10.00±4.88	2.35-19.08
HCO_3^{-} (mgl^{-1})	88.8	21.6	16.8	42.4±23.2	16.8-88.8
SO_4^{2-} (mgl^{-1})	1.81	10.03	0.00	3.95±3.09	0.00-10.03
NO_3^{-} (mgl^{-1})	1.45	1.20	0.00	0.88±0.45	0.00-1.45
PO_4^{3-} (mgl^{-1})	0.25	1.81	1.30	1.12±0.46	0.25-1.81

TDS = Total dissolved solid, TSS= total suspended solid, TS = Total solid, DO = Dissolved oxygen, BOD = Biochemical oxygen demand S.E. = Standard Error

fish pond water observed in this study falls within the standard range of 6.5- 8.0 (Meade, 1989). However, it is regretted seasonal variation was not considered in the study. It has been reported that the level of phosphate in water exceeding 0.03mg/L may be an indication of phosphate pollution of the water body; which may trigger off an excessive growth of algae possibly leading to eutrophication (Richard, 1992). In this study it was observed that the phosphate level of the sampled water is above 0.03mg/l indicating a possible contamination with phosphate compounds.

The dissolved oxygen (DO) in all the ponds studied was between 0 - 4.8 mg/l which is below 5 mg/l recommended standard for fish culture (Meade, 1989).

The values do not agree with 9.3-16.2 mg/l DO reported by Ehiagbonare and Ogunrinde (2010). Low levels DO <0.3 is a lethal concentration that can put undue stress on fish and are often linked to fish kill incidents (Lawson, 1995). However, it has been reported that the African cat fish (*Clarias gariepinus)* can survive in DO levels between 0.0-0.3mg/l because they are obligate air breathers (Department of Water Affairs and Forestry, 1996).

Adequate concentrations of calcium and magnesium are necessary to ensure growth and survival of fish because low levels of calcium reduces disease resistance in fry. The optimal water hardness necessary for fish to thrive is dependent on the species of fish. Most fish grow well over a wide range of hardness values. The total hardness values in this study is between 17.8- 166.5 $CaCO_3$/l and falls within the recommended standard for fish culture (Meade, 1989) and within the permissible 10mg/l hardness for catfish (Department of Water and Forestry, 1996). The values of the other physico-chemical parameters (Table 3) fall within the recommended standard for fish culture (Meade, 1989).

It has been posited that drastic changes in water quality result in poor health and low resistance of fish stock thereby heightening the risks of pathogenic infection, fish diseases or deaths. The physico-chemical analysis of the earthen fish ponds showed that the values fell within the acceptable limit indicating the water quality may not have adverse effect on fish health. However, the detection of pathogenic bacteria that are multi-drug resistant may suggest the need for formulation and implementation of code of practice for fish farmers. This is to ensure appropriate fish management and prevention of transmission of potential pathogens which is important in the part of food safety plan.

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