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Microbiological Analysis of Selected Catfish Ponds in Kano Metropolis, Nigeria

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Abstract: *72 samples of pond water were drawn from 3 fish farms and 1 fish seed multiplication centre in Kano metropolis between the months of July and December, 2012 and were analysed for their bacteriological quality using the International Commission for Microbiological Specification for Food. The results showed means of Aerobic Plate Count (APC) ranged between 1.1x 10^3 – 1.5 x10^5: Escherichia coli Most Probable Technique (MPN) ranged between 6.3 – 8.5, Staphylococcus aureus 1.1 x 10^2 – 2.4 x 10^3, Salmonella paratyphi 1.3 x 10^2 – 1.2 x 10^3, Pseudomonas aeruginosa 1.8 x 10^2 – 2.7 x 10^4, Shigella spp 0.0 – 1.2 x 10^2, Enterococcus faecalis 1.2 x 10^2 – 1.8 x 10^3 and Enterobacter aerogenes 0.0 – 1.1 x 10^2 (P<0.05). Bacterial pathogens isolated and their percentage occurrence were E. coli (43.30%), Staphylococcus aureus (23.21%), Pseudomonas aeruginosa (19.62%), Salmonella paratyphi (7.42%), Shigella spp (0.72%), Enterococcus faecalis (4.52%) and Enteobacter earogenes (1.19%). The findings indicated that the pond water samples were within the acceptable limit except in Bagauda fish seed multiplication centre where the mean bacterial count of Staphylococcus aureus exceeded the limit and the occurrence of Salmonella paratyphi in Bagauda fish seed multiplication centre pond water and Fagam fish pond water where it exceeded the standard of zero tolerance. The occurrence of Pseudomonas aeruginosa, Shigella spp, Enterococcus faecalis and Enterobacter aerogenes in the pond water if not properly checked could endanger both the fish and the ultimate consumers particularly if the fish harvested from these farms are under – cooked.*

Keywords: *Clarias gariepinus, contamination, microbiological specification, Pathogenic bacteria, Pond water,*

I. Introduction

Fish has become increasingly important source of protein and other element necessary for the maintenance of healthy body [1]. The African catfish, Clarias gariepinus has been reared for about 20 years in Africa with mixed success, the total farmed production of these species being only 3.978 metric tonnes or 7.46 mt in Africa [2]. Clarias gariepinus is highly nutritious fish that contains high amount of vitamins, proteins, minerals and little or no saturated fat and is low in carbohydrate [2]. Annual domestic fish supply in Nigeria stands at about 400,000 tonnes. The fishery sector accounts for about 2 percent of national GDP, 40 percent of animal protein intake and a substantial proportion of employment, especially in rural areas. The sector is a principal source of livelihood for over 3 million people [3]. Nigeria is the largest African aquaculture producer, at 15,489 tonnes per year, Egypt (5,645 tonnes) follows Nigeria and then there are only five other countries (Zambia, Madagascar, Togo, Kenya and Sudan) that each produce more than 1000 tonnes [4]. Fish take a large number of bacteria into their gut from water sediment and food [5]. It has been well known that both freshwater and brackish water fishes can harbour human pathogenic bacteria particularly the coliform group [5]. Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish [5]. According to Pillay [6] fish living in natural environment are known to harbour pathogenic enterobacteriaceae. More than 140 invasive bacteria species have been identified in great lakes and other water bodies [2]. Invasion of fish muscles due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish is raised in ponds with faecal coliform, E. coli and Salmonella of greater than 10^3 per ml in pond water respectively [7]. In addition most diseases of humans are caused by opportunistic enteric pathogens, which are prevalent in the rearing environment [8]. Pond water sources are useful for diversified purposes including aquaculture and other related uses at the domestic level. Water is very essential in fish pond, water plays a vital role in the proper functioning of earth ecosystem and also essential for fish and living creatures for metabolism [9]. Water is the most important resource for aquaculture and can be a significant source of contamination [10]. 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. The temperature of water supplied to a fish pond ranges from 25 to 35^0C and this support the growth of microorganisms and fishes in the pond. There were two sources of water, which are borehole and Dam water. Some bacteria coliform groups like E. coli in the pond water are transported from these sources of water or the media of transportation into the ponds. There are several microorganisms found in ponds including bacteria, fungi, algae, protozoa, nematodes and virus [9]. Bacteria has a unique characteristics, they are ubiquitous in every habitation on earth, growing in soil, acidic hot springs, radioactive wastes, water and live bodies of plants and animals [11]. Bacteria are important microorganisms in ponds, some beneficial, others are not. There are various factors affecting the distribution of bacteria in fish ponds which include predatory protozoa present in water. This has significant impact in decreasing the number of bacteria. According to Ugwuba and Chukwudi [12], mortality of fish due to disease and water pollution constitutes problems to aquaculture development in Nigeria. Thus, this study aims at identifying the bacterial isolates of water from some fish ponds in Kano Metropolis and the outcome would serve as a contribution to databank and stakeholders of fish farming.

II. Materials And Methods

The study was conducted in Zogarawa fish farm (Dawakin Tofa Local Government), Bagauda Fish Seed Multiplication Centre (Bebeji Local Government), Fagam fish farm and Khasu integrated fish farm (both in Kumbotso Local Government) of Kano State, which lies between latitude 12^0 00' N and longitude 8^0 31' E (Figure 1). Water samples were collected from four concrete fish ponds stocked with the African catfish (Clarias gariepinus) during the months of July – December 2012 at 6 – 7 GMT time. 100 mls of water were collected in sterile glass ware containers with stoppers. The water samples were transported in ice packs and processed between 2- 3 hours after collection. The microbial load of the water samples from the fish ponds was determined by performing tenfold serial dilution in test tubes containing sterile distilled water. The total viable count was determined using the pour plate technique cultured in duplicates. The plates were inverted to prevent condensation droppings from the lid into the agar and incubated in the incubator at 37^0C for 48 hours. All the glass wares such as petri dishes, conical flask, test tubes, beakers and bijou bottles etc were thoroughly washed and sterilized at 121^0C for about 15-30 minutes in an autoclave. The inoculating loop was sterilized by flaming in the Bunsen burner until it turns hot. Similarly working surface was sterilized by application of disinfectant solution (95% ethanol). Colonies were counted and expressed as colony forming unit per ml. E. coli most probable technique (MPN) was carried out in which 3 tubes containing 9 ml of MacConkey broth with an inverted Durham tube were inoculated with 1ml of sample water to give a dilution of 1:10; From this dilution, 1ml each was transferred to another 3 tubes of MacConkey broth to give a dilution of 1:100; from this second dilution 1 ml was also transferred to another 3 tubes of MacConkey broth to give a dilution of 1:1000. All the tubes were incubated at 37^0C for 24- 48 hours. The number of E. coli positive tubes was determined by MPN index. Sub-culture was carried out until pure isolates were transferred onto Nutrient Agar slant in bijou bottles and kept in a refrigerator at 4^0C to serve as a stock culture for observation of morphological appearance and for the various biochemical tests [13] to determine the identity of the bacterial isolates with reference to Bargey's Manual of Determinative Bacteriology [14].

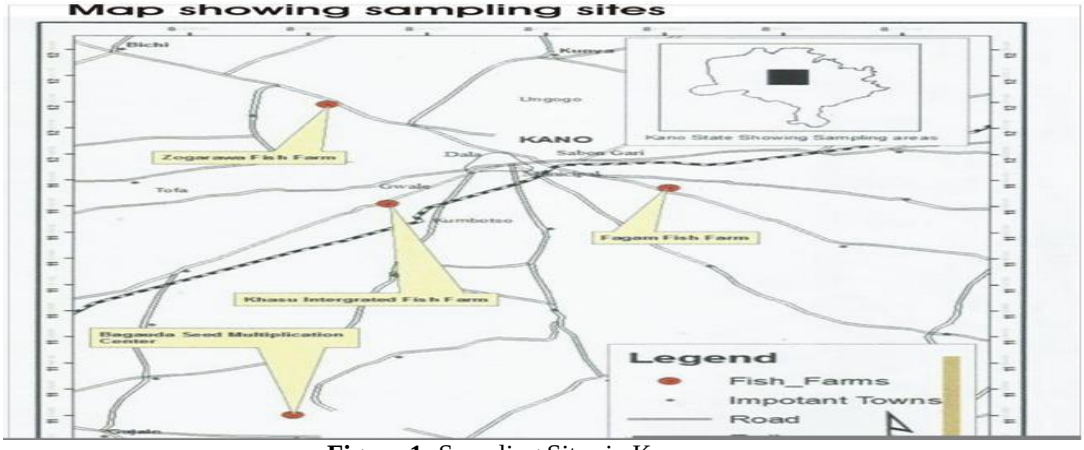

Figure 1: Sampling Sites in Kano

III. Statistical Analysis

One way analysis of variance (ANOVA) using instat 3 statistical software for window version 2003 was used. Bacteriological loads in the pond water were found to be significant ($P<0.05$).

IV. Results

The result in Table 1, show six species in Bagauda fish seed multiplication centre's pond water with the highest bacterial load. APC ($1.5x10^5$), E.coli MPN (8.5), Staphylococcus aureus ($2.4x10^3$), Salmonella paratyphi ($1.2x10^3$), Pseudomonas aeruginosa ($2.7x10^4$), Shigella spp ($1.2x10^2$), Enterococcus faecalis ($1.8x10^3$) and Enterobacter aerogenes ($1.1x10^2$) while three species (i.e the least isolaton) was from Zogarawa fish pond with APC ($1.6x10^3$), E. coli MPN (7.5), S.aureus ($1.3x10^2$) and P. aeruginosa ($1.2x10^3$). Table 2 depicts that E.coli had the highest percentage occurrence (43.30%), followed by Staphylococcus aureus (23.21%) and Pseudomonas aeroginosa (19.62%) while the least was Shigella spp (0.72%). The colonial morphology of the bacterial isolates such as size, margin, elevation, consistency, colour, surface texture and optical features are presented in Table 3, and the biochemical reaction of the isolates such as gram reaction, catalase test, coagulase test, lactose test, indole test, methyl red test, citrate test, voges proskaeur test, oxidase test, urease test, mortality test etc are presented in Table 4.

Table 1: Mean Bacterial Count in Pond Water.

Study Sites	APC	E. coli/MPN	S. aureus	S. para	P. aeru	Shi spp	E. fea	E. aero
Zogarawa	$1.6x10^3$	7.6	$1.3x10^2$	-	$1.2x10^3$	-	-	-
Bagauda	$1.5x10^5$	8.5	$2.4x10^3$	$1.2x10^3$	$2.7x10^4$	$1.2x10^2$	$1.8x10^3$	-
Fagam	$1.1x10^3$	8.2	$1.1x10^2$	$1.3x10^2$	$1.1x10^3$	-	-	$1.1x10^2$
Khasu	$1.2x10^4$	6.3	$1.3x10^2$	-	$1.8x10^2$	-	$1.2x10^2$	-

Mean values on the same row with different superscript are considered significant ($P<0.05$)

Key:

APC = Aerobic Plate Count
E. coli = Escherichia coli
S. aureus = Staphylococcus aureus
S. para = Salmonella paratyphi
P. aeru = P aeruginosa
Shi sp = Shigella specie
E. fae = Enterococcus feacalis
E. aero = Enterobacter aerogenes

Table 2: Percentage Occurrence of Isolates in Pond Water

Isolate identified	Zogarawa	Bagauda	Fagam	Khasu	Total	% occurrence
E. coli	48	54	38	41	181	43.3%
S. aureus	27	31	19	20	97	23.21%
P. aeruginosa	24	40	7	11	82	19.62%
S. paratyphi	-	19	12	-	31	7.42%
Shigella spp	-	3	-	-	3	0.72%
E. faecalis	-	12`	-	7	19	4.54%
E. aerogenes	-	-	5	-	5	1.19%
Total	99	159	81	79	418	100%

Table 3: Colonial Morphology of Bacterial Isolates of Catfish Pond Water Samples

Media used	**Isolate code**	**Size mm**	**Margin**	**Elevation**	**Consistency**	**Colour**	**Surface texture**	**Optical feature**
NA	Cm1	1-2	Irregular	Raised	Viscous	Whitish	Smooth	Translucent
NA	Cm2	1-4	Circular	Flat	Viscous	Whitish	Rough	Transparent
NA	Cm3	1-3	Circular	Raised	Mucoid	Whitish	Smooth	Opaque
EMB	Cm4	1-2	Irregular	Raised	Viscous	Whitish	Rough	Transparent
EMB	Cm5	1-3	Circular	Raised	Mucoid	Milky	Smooth	Translucent
MAC	Cm6	2-4	Irregular	Flat	Viscous	Pale brown	Smooth	Opaque
MAC	Cm7	3-5	Irregular	Raised	Viscous	Pale	Smooth	Opaque

Key:

Cm1 = Staphylococcus aureus
Cm2 = Escherichia coli
Cm3 = Enterobacter aerogenes
Cm4 = Enterococcus feacalis
Cm5 = Pseudomonas aeruginosa
Cm6 = Shigella spp
Cm7 = Salmonella paratyphi

Table 4: Biochemical characteristics of isolates

Sample Code	G. Stain	Cat	Coa	Lact	Ind	Med	Cit	V.p	Ur	Oxi	Mot	Slope	Butt	H^2S	Gas	Probable bacterium
Cm1	+	+	+	-	-	-	-	+	-	-	-	R	Y	+	-	S. aureus
Cm2	-	-	+	+	+	-	-	-	-	-	+	Y	Y	-	+	E. coli
Cm3	-	+	+	+	-	-	+	+	-	-	+	Y	Y	-	+	E. aerogenes
Cm4	+	-	-	+	-	-	-	-	-	-	-	Y	Y	-	-	Ent. faecalis
Cm5	-	+	-	-	-	-	-	-	+	-	-	R	R	-	-	P. aeruginosa
Cm6	-	-	-	-	-	-	-	-	-	-	+	R	Y	-	+	Shigella spp
Cm7	-	+	-	-	-	-	-	-	-	-	-	R	Y	-	-	S. paratyphi

Key:

G. stain = Gram staining
Cat = Catalase test
Coa = Coagulase test
Lact = Lactose test
Ind = Indole test
Met = Methyl-Red test
Cit = Citrate test
V.P = Voges proskaeur test
Ur = Urease test
Oxi = Oxidase test
Mot = Motality test
\- = Negative
\+ = Positive

V. Discussion

Five genera of gram negative bacteria were encountered. These include Escherichia, Shigella, Salmonella, Pseudomonas and Enterobacter. Similarly 2 gram negative bacteria isolated were Enterococcus and Staphylococcus. The APC and E coli MPN of all the four ponds were within the acceptable limit. Staphylococcus aureus count in Zogarawa pond water, Fagam pond water and Khasu pond water were within the acceptable limit but exceeded in Bagauda fish seed multiplication centre. Salmonella paratyphi isolated from Fagam pond water and Bagauda fish seed multiplication centre pond water had zero tolerance in aquaculture. The presence of this species could be from the source, due to excretes in the faeces and urine of infected patients and carriers been wash into the Dam water. Bagauda Dam is a very large unprotected Dam with animal grazing and drinking water around as well as fishing with some irrigation activities. Some parts of the Dam are also used for recreational activities which include boat rides and swimming. All these couple with poor personal hygiene of the fish handlers and poor sanitation of the aquatic environment could be responsible for the contamination of this water. Bacterial isolates from Borehole water were less contaminated when compared with those isolated from the Dam water source (Table 1). This is in agreement with Howard et al. [15] who reported that the ground water such as Borehole when properly constructed and maintained, provide a relatively safer source of raw water in terms of microbial load compared to unprotected water such as rivers, open wells etc. E. coli had the highest percentage of occurrence (43.30%), followed by Staphylococcus aureus (23.21%) and the least was Shigella spp (0.72%). It is natural to expect high occurrence of E. coli because of its high association with faeces of animals or human origin since the fish in the ponds would naturally defecate into the ponds (Table 2). This is in agreement with the investigation made by Egbere et al. [16] on Cat fish ponds in Jos Metropolis, Nigeria. All the organisms isolated in all the pond waters could be implicated for pathogenicity in humans even though their association with specific fish disease has not been successful in Clarias gariepinus [5], probably because the fishes have strong host defence response. The organisms could be involved in the transmission of diseases to humans [17]. Staphylococcus aureus have been implicated in food poisoning outbreak of some food materials, the presence of Staphylococcus aureus is an indication of fish handlers and 80% of them is being harboured by man as normal micro flora [1,18]. Staphylococcus aureus has been associated with different clinical conditions. For example, it is one of the most frequently encountered single bacteria species in hospitals and continues to be the frequent cause of burns and sepsis [19]. It produces pustles, carbuncles, boils and impetigo. It frequently causes septicaemia, osteomyelitis, bacteraemia and otitis [2,19,20]. Pseudomonas aeruginosa could cause general inflammation and sepsis in critical body organs such as lungs, kidneys, urinary tract, which can be fatal because it thrives in most surfaces [2]. E. coli and Shigella spp have been implicated for a number of gastroenteric diseases such as diarrhoea (traveller's disease), dysentery, vomiting, fever, colitis, haemolytic ureamic syndrome with renal failure. Salmonella spp causes salmonellosis which in humans could result in severe typhoid fever (enteric fever) or salmonella fever [16,21] and bacteraemia [22]. Enterococcus spp is a causative agent of dental plagues and scarlet fever [23]. Enterococcus spp has been implicated in human's infections like pharyngitis, scarlet fever and pneumonia [1,18]. Enterobacter aerogenes are opportunistic pathogens, associated with urinary infections, wound infections, and septicaemia, especially in persons already in poor health [13]. Therefore, the pond water did not conform to the specification that no pathogen should be found in catfish ponds [24]. The mere presence of microbes in pond water in small number is usually not harmful but their unrestricted growth may render the pond water harmful to the catfish and also to the ultimate consumers. The bacteriological examination of fish pond water is very important to detect the presence of bacteria that might constitute health hazard and death of fish in the fish pond. This can serve as a guide to monitor and protect fish ponds. It is also necessary in order to detect the kind of bacteria being transferred from water source to the fish pond.

VI. Conclusion And Recommendation

It can be concluded that the catfish ponds water from Zogarawa and Khasu were less contaminated compared to Bagauda and Fagam which had Salmonella paratyphi. Pond water contaminated with these pathogenic bacteria could endanger consumers particularly if fish harvested from these ponds are under-cooked. It is therefore recommended that the environment where fish ponds are located should be protected from pollutants and weeds which can harbour microorganisms that can find their way into fish ponds by themselves or by passive process through wind, rainfall, etc. Dam water supply to the fish pond should be taken and examined in the laboratory for its microbiological quality before stocking, this would give insight to the possible presence of certain types of microorganisms, hence provide enabling environment for aquaculture purposes. Fish handlers with open wounds should avoid contact with water from the fish ponds and fish should be properly cooked with heat before consumption.

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