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Effect of Pathogenic Bacteria on Cat Fish (*claria gariepinus)*

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Abstract: A study on the quality status of cultured clarias gariepinus from 4 Fish Farms was carried out. The International Commission of Microbiology Specification for Food (ICMSF 2007) technique was employed to test samples for APC, Escherichia coli MPN estimation, and enumeration of Staphylococcus aureus, enumeration of Salmonella spp and Enumeration of Vibrio parahaemolyticus. The APC ranged from 1.1.x103 – 1.5x105 and E. coli MPN ranged from 6.3 – 10.4 both were within the prescribed limits. Staphylococcus aureus enumeration of 2.4 x 103, 2.1x104 and 2.0x104 for pond water, skin and gill of Clarias gariepinus respectively in Bagauda fish seed multiplication center exceeded the limit of < 103. Salmonella paratyphi occurrence from Baguda fish seed multiplication center and Fagan fish farm range was 1.2x102-2.1x103 which exceeded the limit of zero stipulated by ICMSF (2007). Pseudomonas aeruginosa (1.1x102-2.7x104), Enterococcus feacalis (1.1x102-1.8x103), Enterobacter aerogenes (1.1x102-1.2x102) and shigella sp (1.2x102) encountered are pathogens of public health importance which exceeded the limit of zero tolerance (WHO 2003) and (EPA 2003).. Statistical analysis (ANOVA) showed significant difference (P<0.05) between bacterial loads in pond water, skin and gill of Clarias gariepinus.

Keywords: Pathogenic, Bacteria, Escherichia, Clarias gariepinus, Vibrio parahaemolyticus.

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INTRODUCTION

The ability of water to harbour microbial pathogens and cause subsequent illness is well documented for both developed and developing countries (Okonko *et al.,* 2008). Water related diseases continue to be one of the major health problems globally (Adebayo-Tayo *et al.,* 2011a, b). WHO 1986 estimates that some 30,000 people die every day from water related diseases like typhoid fever, cholera, bacillary dysentery and gastroenteritis (Bichi *et al,* 2002) Okonko *et al,* (2008, 2009) reported that both bacteria and fungi are common flora of frozen fish and fish related products during packaging. It is estimated that 80 of all illnesses are linked to using water of poor microbiological quality (Okonko *et al,* 2008, 2009). The microbial composition of fish depends on the microbial counts of the water in which they live. However, the fresh and internal organs of freshly caught healthy fish from tropical and temperature water are normally sterile because the scale and slime covering the fish serve as biological barriers to the entry of microorganisms (FEHD, 2005). The ponds and rivers that harbour the fish may be the source of contaminants due to the indiscriminate deposition of human, animal excreta and other environmental wastes into natural water and land especially during the rainy season, as feacal matter from various sources is washed from contaminated land into different water bodies (Emikpe *et al,* 2011). Free roaming animals and pets especially dogs also contribute to feacal contamination of surface water. Run off from roads, parking lots and yards can carry animal wastes into natural watercourse and ponds (Emikpe *et al,* 2011). Birds can also be a significant source of bacteria. Swans: Geese and other water fowl can all elevate bacteria counts in water bodies and ponds (Doyle & Ericson cited in Adedeji *et al*., 2012).

Fish take a large number of bacteria into their guts from water segment and food (Emikpe *et al.,* 2011). It has been well known that fresh and brackish water fishes can harbour human pathogenic bacteria particularly the coliforms group (Emikpe *et al.,* 2011). Faecal coliform in fish demonstrate the level of pollution in their environment because coliform are not named flora of bacteria in fish (Emikpe *et a.,* 2011). Fish contamination can also be linked to raw materials, personnel, processing tools such as forklifts through leakage, openings in buildings and pests. Some pathogens may even become established in the processing plants from niches where they can survive

for long period of time (Adebayo-tayo *et al.,* 2012b). The tissue of a healthy fish is normally considered sterile until bacteria inversion that leads to spoilage. According to Adams and Moses (2008), the normal bacterial load of the surface slime of fish can range from 10^2 -10^7 cfu/cm^2 and the gills and intestines can range up to 10^3-10^7 cfu/lg respectively.

The contaminating fish observed in most studies may result from rupturing fish intestine during poor processing or inadequate washing as intestinal micro flora of human or animal origin are the causative agents for food spoilage (Emikpe *et al.,* 2011). The higher density of total aerobic bacteria found in the skin and stomach of fishes might be due to quick proliferation after catching and during transportation and storage (Emikpe *et al.,* 2011). Preservation in low quality ice, handling with contaminated hands could also be responsible for higher density of aerobic bacteria (Emikpe *et al.,* 2011). In addition, bacteria may be found on the skin, chitinous shell, gills as well as the intestinal tracts of fish and shell fish. If subsequent handling is improper and there is inadequate pathogens reduction step (e.g cooking) afterwards the level of bacteria in the final product may increase to such an extent that may present health risk to consumer (FEHD, 2005). Even though epidemiological evidence on outbreak of food borne disease is scarce there is an indication that the food could be contaminated to unsafe level at the point of consumption with air flora and other microorganisms from handlers, equipment/utensils and the raw food materials (Edema *et al.,* 2008). Fish disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and the contraction of zoonotic disease by the handler and final consumer of the affected fish (Emikpe *et a.l,* 2011). Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other food (Azubuike, 2019). Fish and shellfish not only transmit diseases to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxication (Emikpe *et al.,* 2011). Therefore, maintenance of good quality is of utmost importance in production and trade of fishery products, however, most of current quality control techniques are time consuming and cumbersome (Yagoub, 2009; Adebayo-Tayo *et al.,* 2012b). Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food borne illness (Ajao & Atere, cited in Adedeji *et al.,* 2012). However, contamination offish may occur from fish handlers and retailers who sell these items to the public for consumption (Adebayo-Tayo *et al* cited in Adedeji *et al.,* 2012).

Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, drug treatments, undernourishment, fluctuating temperature and poor water quality (Azubuike, 2016). All these factors can impose a considerable stress on the homeostatic mechanisms of fish rendering them susceptible to a wide variety of pathogens (Chioma *et al.,* 2010). These bacteria are normally free living inside the water but are capable of adopting a pathogenic role under the aforementioned circumstances (Harishi *et al.,* 2003). Many of these organisms are saprophytes living on dead organic matter such as plants and animals but bacterial infection in fish results because of changes in the bacteria/fish relationship (Chioma *et al.,* 2010). Fish safety is of increasing importance in an aquaculture industry and the quality of fish varies from producer to another. These have necessitated this study to create awareness among both the producers and the consumers about the existing situation and to protect the consumer's health and right.

AIMS AND OBJECTIVES

This research is aimed at determining the effect of pathogenic bacteria on cultured *clarias gariepinus* in some selected fish farms in Kano State, Nigeria. The specific objectives of this study are to:-

a. estimate the level of bacterial contamination of pondwater

b. estimate the level of bacteria contamination of gills and skin of fish

c. observation of wellbeing (conditioned factor) in the fishes

Commented [H1]:

MATERIALS AND METHODS

Study Area

The study was conducted in Dawakin Tofa Local Government Area (Zogarawa fish farm), Bebeji local government area (Bagauda fish seed multiplication center) and Kumbotso local government area (both Fagam fish farm and Khasu integrated fish farm) of Kano State. Kano Geographical Profile lies between latitude 12^0.00'N and longitude $8^0$31'E. With the population density of Nine Million, Three Hundred and Eighty Three Thousand, Six Hundred and Eighty Three people (9,383,683) (NPC 2006). The State is characterized by the two seasons. The rainy season which last from May to September and dry season which last from October to April. The mean annual temperature is between 16-47^{0}C and the mean annual rainfall ranges from 700-1160mm (Alhassan *et al.,* 2008).

Sample Size Determination

Random sampling method was used and the sample size was determined by the following formula:

$n = z^2pq\text{-}d^2$ (Lwanga, 1991) where:

n = minimum sample required
z = standard under normal deviate corresponding to 5% level of significance.
The value obtained from the normal distribution table is 1.96.
p = prevalence of bacteria flora from healthy *C gariepinus* and their
antimicrobial resistance pattern (Efuntoye *et al,* 2012)
q = Complementary probability to P = 1-P
d = decision of estimate (5%=0.005)
$n = (1.96)^2 x\ 0.09 \times 1\text{-}0.09\text{-}(0.005)^2$
$n = 3.842 \times 0.09 \times 0.9K\ (0.005)^2$
$n = 0.3146598\text{-}(0.005)^2$
n= 125.86392 -126
Adding 5% attrition
126x5-100=6.3
n =126+6
n=132
Total samples size is 132÷4=33, thus, 33 fish samples were taken from each fish farm.

Sample collection

Live cultured *C. gariepinus* were collected from 3 ponds of commercial farms and 1 fish seed multiplication centre. 132 samples of *C. gariepinus* and 72 samples of pondwater were randomly collected from different locations and analysed. Fish were caught by a cast net and hand net. Sampling was done between 7.00-9.00am in each occasion at a periodic interval of seven days for six months. Fish samples were transported in clean polythene bags directly to postgraduate laboratory of the Biological Sciences Department, Bayero University Kano within two hours of sampling.

Sample preparation

Bacterial isolates from each sample were obtained from pond water, skin and gill of C. gariepinus. $1cm^2$ of skin and 1g of gill was macerated aseptically in separate tubes and was swirled in 10ml distilled water. The stock solution was serially diluted tenfold, each tube containing 9ml buffered peptone water and was placed in a test tube rack. 1ml of the stock solution was transferred into the first test tube and was labelled 10^{-1} dilution. From the first test tube, 1ml was transferred into the second test tube labelled 10^{-2} dilution. 1ml of the second test tube was transferred to the third test tube and was labelled 10^{-3} from the third test tube, 1ml was transferred to the fourth test tube and was labelled 10^{-4}. 1ml of the fourth test tube was transferred to the fifth test tube and was labelled 10^{-5} and 1ml was transferred from the fifth test tube to the sixth test tube and was labelled 10^{-6} dilution. O.lml of each diluent was inoculated into two petri dishes and about 20ml of molten nutrient agar was poured on the petridishes. The researcher replicated this process using MacConkey agar and Salmonella Shigella agar. The plates were mixed by swirling and the medium was allowed to cool and solidified undisturbed. The plates were appropriately labelled with sample no, sample site, date, media used and the dilution factor inoculated. The plates were incubated aerobically in an incubator at 37^0C for 24-48 hours.

Determination of Bacterial Count.

The total bacterial count was determined according to APHA (1995). Visible and distinct colonies on the plates containing 30-300 colonies were counted by dividing the bottom side of the culture plates into four quadrant. The counting was done quadrant by quadrant which was summed up and the number obtained was multiplied by the dilution factor and divided by the quantity of inoculum to get the number of colony forming units per ml.

Microbiological Assay

The methods recommended by International Commission of Microbiological Specification for Food (ICMSF, 2007) to determine the quality of fish was employed. It involved assaying for certain microorganisms of significance in fish such as:-

a. Enumeration of Mesophilic aerobic bacteria
b. Enumeration of *E. coli*
c. Enumeration of *Staphylococcus aureus*
d. Enumeration of *Salmonella spp*
e. Enumeration of *Vibrio paraheamolyticus*

Enumeration of Mesophilic Aerobic Bacteria

0.1ml of the sample dilution was inoculated in each of the appropriately marked duplicates dishes.
About 20ml of the molten nutrient agar was poured into each of the petridishes and this was mixed and allow to solidify undisturbed, the petridishes were appropriately labelled and incubated at 37°C for 24 -48 hours. Colonies were counted. Pure cultures were obtained by subculturing on freshly prepared nutrient agar.

Enumeration of *E. coli*

Presumptive test: The presumptive test for coliforms by the most probable number technique (MPN) was carried out in which three tubes containing 9 ml of MacConky broth with an inverted Durham tube were inoculated with 1 ml of the sample water to give a dilution of 1:10. From this dilution, 1 mL of each was transferred to another 3 tubes of MacConky broth to make a dilution of 1:100. The same procedure was followed to give a dilution of 1:1000. All the test tubes were incubated at 370 °C for 24-48 hours. Tubes with acid production were shown by a change in colour of MacConky broth from purple to yellow, and gas production by the collection of a bubble in the Durham tube was recorded as positive for

the presence of coliform. Negative tubes were recorded (FAO, 2003).

Enumeration of Staphylococcus aureus

0.lml of each dilution was inoculated into petri dishes in duplicates, and about 20 ml of molten Mannitol salt agar was poured and allowed to solidify undisturbed and labeled. The plates were incubated at 37°C for 24–48 hours. The plates containing 30–300 golden yellow colonies were used, and gramme staining and biochemical tests such as catalase, coagulase, etc. were carried out.

Enumeration of Vibro Parahaemolyticus

0.1 ml of each dilution was inoculated into petri dishes in duplicates; about 20 ml of molten macConkey agar was poured into the petri dishes and allowed to solidify undisturbed and labeled. The plates were incubated at 37°c for 24-48 hours and examined for small, non-lactose fermenting colonies. There were no Vibrio spp detected in all the samples examined.

Biochemical tests Indole test

The Indole production test was used for the differentiation of gramme-negative cteria. It works on the principle that some bacteria are able to breakdown the amino acid tryptophan present in peptone water to release indole. The reagent used was Kovac's reagent (Cheesbrough, 2010). the test organism was inoculated in a bijou bottle containing 3 ml of sterile peptone water, the peptone water cultured was incubated at 370C for 48 hrs. 0.5 ml of Kovac's reagent was added to the overnight peptone water culture and shaken gently. The appearance of a red-coloured ring indicates a positive indole test. E coli indicated a positive indole test (Cheesbrough, 2010).

Methyl-red test

Methyl- red test was carried out to differentiate Enterobacteria. It detects the production of sufficient acid during the fermentation of glucose, different organisms produce different end products at different pH levels. Some bacteria with extended incubation times are able to maintain a pH level below 4.5. This was demonstrated by the change in colour of the methyl red indicator, which was added during the incubation period (Cheesbrough, 2000). This test was carried out by inoculating the medium with a young culture of the organism on nutrient agar. The inoculated medium was incubated at 35 C for two days. Five drops of the indicator were added to the culture, and the presence of the red colour indicates a positive methyl-red test, and a lack of the colour change indicates negative methyl red.

Voges Proskeaur Test

This test was employed to differentiate some Enterobacteria. The test was carried out by inoculating about 2ml of medium with the test organism. The inoculated medium was incubated at 37°C for 48 hours. 0.5 ml of 6% a-naphthol, followed by 0.5 ml of 16% KOH, was added, and the inoculated medium was left at room temperature for one hour. A red colour change indicates a VP reaction, while no colour change indicates a negative VP test. Salmonella spp indicated a negative VP test.

Citrate Utization Test.

This test is one of the most important tests used in the identification of Enterobacteria. Its principle is based on the ability of an organism to utilise citrate as the only source of carbon and ammonium as the only source of nitrogen. The organism metabolizes the citrate to produce acetoin and CO2. The test was carried out by making a light suspension of the organism, which was stabbed and inoculated with a straight wire loop in simmon citrate medium (Cheesbrough, 2010). A growth of blue colour in the simmon's agar indicate a positive result, it showed that citrate has been utilized. Salmonella spp showed a negative result to citrate test

Coagulase test

A drop of saline was placed on a glass slide and the test organism was emulsified on it to give a milky suspension (Cheesbrough, 2010), a drop of rabbit plasma was added to the suspension. The plasma was stirred using a wire loop, clumping of the organism was checked and those that showed clumping indicate positive result, eg Staphylococcus aureus.

Catalase test

This is to differentiate those bacteria that produce the enzyme catalase, such as Staphylococcus aureus from non-catalase producing bacteria such as Streptococci. 2 drops of hydrogen peroxide were added to a glass slide containing the test organism. The author immediately placed it on a petridish and covered it. Bubbles observed through the lid indicate a positive test (Cheesbrough, 2000).

Urease Test

The test is important in distinguishing enterobacteria. The principle behind it is based on the fact

that some organisms produce urease enzymes. The enzyme breaks down urea (by hydrolysis) to give ammonia and carbohydrate. With the release of ammonia, the medium becomes alkaline, as shown by the change in colour of the indicator from pink to red (Cheesbrough, 2000). The author inoculated the test organism in a test tube containing sterile biomark urea agar. The culture obtained was incubated at 370 °C. Salmonella spp showed a negative result to the urease test (no pink colour change).

Oxidase Test

This test is used to assist in the identification of Pseudomonas which produces the enzyme cytochrome oxidase. A piece of paper was placed in a clean petri dish, and 2 drops of freshly prepared oxidase reagent were added. The author used a piece of matchstick to extract a colony of the organism, which was then smeared onto the filter paper. A positive oxidase test showed a blue-purple colour within 10 seconds (Cheesbrough, 2010). Pseudomonas aeruginosa indicated a positive result.

Motility Test

5 ml of nutrient broth (8.0g / L) was dispensed in test tubes and left to settle in vertical position. The test organism was inoculated with straight needle i.e. stabbed down the center. After incubation at 370C, observations at 24 -48 hours were made. Diffused hazy growth that spreads throughout the medium rendering it slightly opaque indicates a positive result, while growth confined to the stabbed line with sharp defined line shows a negative result (Shields & Cathcar, 2011).

Characterization on MacConkey Agar

The agar was dissolved at a concentration of 5.2g/100ml of distilled water. Sterilization by autoclaving was done at 1210C for 15 minutes. When the medium had cooled down to about 50–550 °C, it was dispensed aseptically in sterile petridishes, the medium was dated and given batch numbers. The test organism was inoculated onto the medium using sterile wire loops and was incubated at 370C. MacConkey agar differentiates between lactose'-fermenting ' organisms and non-lactose fermenting organisms. E coli were a lactose fermenter while Salmonella spp was a non-lactose fermenter. E coli and other lactose fermenters produce pink colonies (Cheesbrough, 2000).

Characterization on Kligler iron agar

The medium was prepared at a concentration of 5.5g in every 100ml of distilled water. The medium was sterilised by autoclaving at 1210C for 15 minutes, it was gently poured into sterile test tubes and allowed to cool and solidify in a slope position. The test organism was inoculated into the medium and incubated at 370C (Cheesbrough, 2000). Dates and batch numbers were given to the test tubes. Salmonella produce a pink, red slope and yellow butt with cracks underneath the agar due to gas production from glucose fermentation.

Statistical Analysis

Date for the bacteriological loads of pond water, skin and gill of C. gariepinus were analyzed determined using one-way analysis of variance (ANOVA). Using Instate 3 statistical software for Windows 2003.

RESULT

Table 1: Mean Bacterial Count in Pond Water

Study Sites	APC	*E.coil/MPN*	*S.aureus*	*Sal sp*	*v.para*	*p.earu*	*Shi sp*	*E.fae*	*E.aero*
Zogarawa Fish Farm	$1.6x10^3$	7.6	1.3x102	-	-	$1.2x10^3$	-	-	-
Bagauda Fish Seed Multiplication Center	$1.5x10^5$	8.5	2.4x103	1.2x103	-	$2.7x10^4$	$1.2x10^2$	$1.8x10^3$	-
Fagam Fish Farm	$1.1x10^3$	8.2	1.1x102	1.3x102	-	$1.1x10^3$	-		$1.1x10^2$
Khasu. Integrated Fish Farm	$1.2x10^4$	6.3	1.3x102	-	-	$1.8x10^2$	-	$1.2x10^2$	-

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

Key

APC – Aerobic Plate Count
E.coli/MPN-*E.Coli* / most probable number
S.aureus – Staphylococcus aureus
Sal; sp – Salmonella specie
V. Para – Vibrio Parahaemolyticus
P. aeru – Pseudomonas aeruginosa
Shi sp – shigella sp
E.fae – Enterococcus faecalis
E.earo – Enterobacter aerogenes

Table 1: present mean bacterial loads of pond water. The result of this table shows that the Bagauda fish seed multiplication centre pond water had the highest mean of bacterial loads. APC ($1.5 x10^5$), *E coli* MPN estimation (8.5), *Staphylococcus aureus* ($2.4x10^3$), *Salmonella paratyphi* ($1.2x10^3$), *Pseudomonas aeruginosa* ($2.7xl0^4$), *Shigella sp* ($1.2xl0^2$), *Enterococcus feacalis* ($1.8x10^3$) and *Enterococcus aerogenes* ($1.1x10^2$) from Fagam fish farm. On using analysis of variance (ANOVA) bacterial loads in the pond water from the various farms were found to be significant (P<0.05).

Table 2: Mean Bacterial Count in Skin of *Clarias gariepinus*

Study Sites	APC	*E.coil/MPN*	*S.aureus*	*Sal sp*	*v.para*	*p.earu*	*Shi sp*	*E.fae*	*E.aero*
Zogarawa Fish Farm	$1.8x10^3$	8.2	$1.4x10^2$	-	-	$1.8x10^3$	-	-	-
Bagauda Fish Seed Multiplication Center	$1.7x10^4$	10.4	$2.1x10^4$	$2.1x10^3$	-	$2.2x10^3$	-	-	-
Fagam Fish Farm	$2.2x10^3$	8.7	$1.3x10^2$	1.8x10	-	$1.6x10^4$	-		-
Khasu. Integrated Fish Farm	$2.0x10^3$	7.5	$1.2x10^2$	-	-	$1.1x10^2$	-	-	-

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

Key

APC – Aerobic Plate Count
E.coli/MPN-*E.Coli* / most probable number
S.aureus – Staphylococcus aureus
Sal; sp – Salmonella specie
V. Para – Vibrio Parahaemolyticus
P. aeru – Pseudomonas aeruginosa
Shi sp – shigella sp
E.fae – Enterococcus faecalis
E.earo – Enterobacter aerogenes

Table 2: presents the mean bacterial loads of skin of *Clarias gariepinus.* The results of this table indicated that the highest bacterial loads was from the skin of *Clarias gariepinus* collected from Bagauda fish seed multiplication center with APC ($1.7xl0^4$), *E coli* MPN (10.4), *Staphylococcus aureus* ($2.1xl0^4$), *Salmonella paratyphi* ($2.1xl0^3$), but the highest *Pseudomonas aemginosa* was from Fagam fish farm ($1.6xl0^4$). On using one way analysis of variance (ANOVA), bacteria among the skin from various farms were found to be significant ($P<0.05$).

Table 3: Mean Bacterial Count in Gill of *Clarias gariepinus*

Study Sites	APC	*E.coil/MPN*	*S.aureus*	*Sal sp*	*v.para*	*p.earu*	*Shi sp*	*E.fae*	*E.aero*
Zogarawa Fish Farm	$1.2x10^4$	7.1	$1.2x10^2$	-	-	$1.6x10^2$	-	-	-
Bagauda Fish Seed Multiplication Center	$1.3x10^3$	9.1	$2.0x10^4$	$1.2x10^2$	-	$1.7x10^4$	-	$1.7x10^2$	-
Fagam Fish Farm	$1.9x10^3$	7.5	$1.2x10^2$	$1.7x10^2$	-	$1.2x10^2$	-		$1.2x10^2$
Khasu. Integrated Fish Farm	$1.3x10^4$	6.7	$1.7x10^2$	-	-	$1.5x10^2$	-	$1.1x10^2$	-

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

Key

APC – Aerobic Plate Count
E.coli/MPN-*E.Coli* / most probable number
S.aureus – Staphylococcus aureus
Sal; sp – Salmonella specie
V. Para – Vibrio Parahaemolyticus
P. aeru – Pseudomonas aeruginosa
Shi sp – shigella sp
E.fae – Enterococcus faecalis
E.earo – Enterobacter aerogenes

Table 3: presents the mean bacterial loads of gills of *Clarias gariepinus.* The result of this table show that the gills of *C. gariepinus* from Khasu integrated fish farm had the highest APC ($1.3X10^4$), highest *E coli* MPN was from Bagauda fish seed multiplication center (9.1), highest loads of *Staphylococcus aureus* ($2.0xl0^4$) was also from Bagauda fish seed multiplication center, highest loads *of Salmonella paratyphi* ($1.7xl0^2$) was from Fagam fish farm, *Pseudomonas aeruginosa* highest loads was from Bagauda fish seed multiplication center ($1.7xl0^4$),highest loads of *Enterococcus feacalis* ($1.7xl0^2$) was also from Bagauda fish seed multiplication center and the highest loads of *Enterobacter aerogenes* ($1.2xl0^2$) was from Fagam fish farm. On using one way analysis of variance (ANOVA) of the bacterial loads of gills between the study sites show there is significant difference ($P <0.05$).

Table 4: Distribution of Pathogenic Bacteria in Pond Water, Skin and Gill of *C.gariepinus*

Isolate	Pond Water	Skin	Gill
E. coli	+	+	+
S. aureus	+	+	+
P. aeruginosa	+	+	+
S. Paratyphi	+	+	+
Shigella sp	+	-	-
E. faecalis	+	-	+
E. aerogene	+	-	+

Key: + = present = absent

Table 4: presents the distribution of pathogenic bacteria from pond water skin and gill of *C. gariepinus.* The result depicts that *E coli, S. aureus, P.aeruginosa, S. paratyphi, Shigella sp, E. feacalis* and *Enteobacter aerogene* were present in pond water of the various fish farms. *E coli S. aureus, P. aeruginosa* and *S. Paratyphi* were present on skin, while *shigella sp* was the only specie absent on the gills.

Table 5: Percentage of Bacteria isolated in Pond Water, Skin and Gill of *C. gariepinus* from Four Fish Farm

Isolate identified	Zogarawa Farm	Bagauda Farm	Fagam Farm	Khasu Farm	Total	% occurrence
E. coli	53	43	39	51	186	45.04
S. aureus	27	35	19	20	101	24.46
P. aeruginosa	24	22	7	13	66	15.98
S. Paratyphi	-	19	12	-	31	7.51
Shigella sp	-	3	-	-	3	0.73
E. faecalis	-	12	-	7	19	4.59
E. aerogene	-	-	7	-	7	1.69
	104	134	4	91	413	100%

Values are percentages of isolates ($P<0.05$)

Table 5: present the percentage (%) occurrence of bacterial isolates from pond water, skin and gill of C. *gariepinus* from 4 fish farms. The result of the percentage occurrence of isolate show that *E coli* were the most frequently encountered isolate (45.04%).

DISCUSSION OF FINDINGS

The mean bacterial loads of pond water from 4 fish farms show that the bacterial load was less in borehole water (Zogarawa fish farm, Fagam fish farm, and Khasu integrated fish farm range from I.lxl0^2-1.2xl0^4) and high in dam water (Bagauda fish seed multiplication centre ranges from 1.2x10-1.5x10). The bacterial loads from Bagauda fish seed multiplication centre pond water were the highest. APC (1.5 x 105); *E. coli* MPN (8.50); *S. aureus* (2.45 x 10^3); *S. paratyphi* (1.2 x 10^3); *P. aeuginosa* (2.7 x 10^4); *Shigella sp. (* 1.2 x 10^2), *Enterococcus faecalis* (1.8 x 10^3). This finding agrees with that of Howard et al (2003), who reported that groundwater sources such as boreholes, when properly constructed and maintained, provide a relatively safer source of raw water in terms of microbial load compared to unprotected water sources such as rivers, open wells, etc.

The high contamination was due to the source of water from Bagauda Dam. Bagauda Dam is a very large, unprotected dam with animal grazing and drinking water around it, as well as fishing and some irrigation activities. Some parts of the dam are also used for recreational activities, which include boat rides and swimming. All these, combined with poor personnel hygiene and poor sanitation of the aquatic environment, could be responsible for the contamination. It should be noted that all of the organisms isolated could be implicated in pathogenicity, and since all the ponds contained at least three of these pathogens, One could rightly state that none of the water in the ponds conformed to the specification that no pathogen should be found in the catfish ponds (Paul & Loader, 2007).

The mean bacterial loads in $1cm^2$ of skin of *C. gariepinus skin* from 4 fish farms show that APC, *E coll* MPN of C. *gariepinus* skin from all the farms were within the acceptable limit. *Staphylococcus qureus* from the Bagauda fish seed multiplication centre exceeded the

limit. *Salmomella paratyphi* found on the skin of *C. gariepinus* from the Bagauda fish seed multiplication centre and Fagam fish farm is not acceptable, as the agency stipulated zero tolerance. *Pseudomonas aeruginosa* was found in all the farms, which is not also acceptable to both the WHO (2003b) and the EPA (2003). Vibrio parahaemolyticus was not found. The result shows that the skin favours microbial growth. It is prone to microbial contact in water, the common environment for microorganisms and fish.

The mean bacterial loads in Ig of gills of *C. gariepinus* from 4 fish farms show that the APC and *E coli* MPN of the gills from all the farms were within the permissible level. *Staphylococcus aureus* from the Bagauda fish seed multiplication centre gill exceeded the limit. *Salmonella paratyphi* was found in the Bagauda fish seed multiplication centre and the Fagam fish farm, and its presence is not acceptable at all. *Pseudomonas aeruginosa* was found in the gill of *C. gariepinus* in all the farms. *Enterococcus faecalis* was found in the Bagauda fish seed multiplication centre and the Fagam fish farm. *P. aeruginosa, E. faecalis,* and *E. aerogene* exceeded the stipulated zero tolerance by the WHO (2003) and EPA (2003). No *Vibrio parahaemolyticus was* found.

The distribution of pathogenic bacteria from pond water, skin, and gills of *C. gariepinus* revealed that the bacteria species isolated from skin and gills of *C. gariepinus* is a reflection of the bacteria isolated from pond water; the bacteria flora of fish depicts the bacteria flora of the water environment. This is in agreement with observations made by Obiajaru and Ogbulie (2006) in Imo State, Nigeria.

Shigella sp. was isolated from Bagauda fish seed multiplication centre pond water only but was not found on the skin and gill of *C. gariepinus.* This is because *Shigella sp* infects only humans (Cheesbrough, 2010). The source of *Shigella sp* in the pond water from the Bagauda fish seed multiplication centre could be through the fecal-ororal route with poor sanitation, unhygienic conditions, or infected food handlers. Houseflies help to transfer *Shigella* from faeces to food (Cheesbrough 2010). 7 species: *E. coli, S. aureus,* and *Shigella sp* were isolated from the pond water of the various 4 fish farms. 4 bacterial species were isolated from the skin, viz: *E. coli, S. aureus, P. aeruginosa,* and *S. paratyphi.*

The mere presence of microbes in foods in small numbers is usually not harmful, but their unrestricted growth may render the food unfit for consumption (Mukhtar *et al* 2001; WHO, 2007).

The percentage (%) occurrence of pathogenic bacteria isolated from 4 fish farms revealed that *E. coli* had the highest frequency of occurrence (45.04%). The high occurrence of *E. coli* is due to its high association with faeces of animal or human origin, since the catfish in the ponds would naturally defecate into the pond water. This is in agreement with the investigation made by Egbere *et al.* (2010) in Jos, Plateau State. Nigeria.

The observed fish's wellbeing (conditioned factor) showed that generally, fish were affected by these pathogenic bacteria. The areas mostly affected are the gills, which are richly supplied with blood vessels, and the skin, which is also susceptible to infection (Azubuike, 2019). Because of the effect of these pathogenic materials on these fish, it is expected that the nutritional values will; be drastically reduced, and as such, it is advised not to be consumed (Azubuike, 2016). Thus, it is advised that the bodies of water where these fish were collected should be treated properly before restocking.

CONCLUSION

The findings of this study show that most of the physicochemical parameters are within the prescribed water quality standard. The APC and *E. coli* of all the fish farms were within the prescribed standard, but the *S. aureus* exceeded the limit in the Bagauda fish seed multiplication center. *Salmonella paratyphi,* found in the Bagauda Fish Seed Multiplication Centre and Fagam, exceeded the limit. *P. aeruguiosa, E. faecalis, E. aerogenes,* and *Shigella sp* exceeded the zero tolerance; therefore, consumers are at risk of buying contaminated fish harvested from these farms.

RECOMMENDATIONS

1. Water used for fish farming should be treated before being introduced to the ponds to reduce the degree of contamination, especially the dam water from the Bagauda fish seed multiplication centre.
2. Fish handlers should avoid contact with fish when they have skin injuries.
3. There should be regular disinfections of catching gear and working equipment.
4. Caught fish should be briefly immersed in brine water to reduce the microbial load before being stored at a cold temperature or sold to the public.
5. Fish should be property processed with heat to avoid ingesting pathogens.

REFERENCES

Adams, M. R., & Moses, M.O. (2008). *Food microbiology (third edition).* The Royal society of chemistry. Cambridge, UK. 2008, 179pp.

Adebayo-Tayo, B.C., Adegoke, A.A., & Akinjogunla, OJ. (2009). Microbial and physico-chemical quality of powdered soy milk samples in Akwa Ibom, South-Southern Nigeria. *African Journal of Biotechnology* 8(13):3066-3071.

Adebayo-Tayo, A.C., OduN.N., Micheal, M.U., & Okonko, I.O. (2012a). Multi drug resistant (MDR) organisms isolated from seafoods in Uyo, south-southern Nigeria. *Nature and Science, 10 (3), 61-70*

Adebayo-Tayo, A.C., Odu, N.N., Anyamele, L.M., Igwiloh NJPN., & Okonko I.O. (2012b). Microbial quality of frozen fish sold in Uyo Metropolis. *Nature and Science* 10(3), 71-77

Adebayo-Tayo, B.C., Odu, N.N., Igwiloh, NJPN & Okonko I. O.(2012d). Microbiological and physico- Chemical level of fresh catfish *(arius hendelotic}* from different markets in Akwa Ibom State, Nigeria. *New York Science.* 5(4), 46-52.

Adebayo-Tayo, B.C., Okonko, I.O., Esen, C.U., Odu, N.N., Onoh, C.C., & Igwiloh NJPN (201la). Incidence of potentially pathogenic *Vibrio spp* in fresh seafood from Itu creek in Uyo, Akwa Ibom State, Nigeria *World Applied Science Journal,* 15(7), 991.

Adedeji, O.B., Okerentugba, P.O., Innocent-Adiele, H.C., & Okonko, I.O. (2012). Benefits, Public health hazard and Risk associated with fish consumption. *New York Science Journal,* 5(9), 33-61. Available online @:http: //www.sciencepub.net/new York.

Alhassan, A.J., Imam, A.A., & Yakasai, H.M. (2008). Quality assessment of Sachet waterpacked around Kano *Metropolis.BAJOPAS* l(l):83-87

APHA. (American Public Health Association) (1998). *Standard Methods for Examination of Water and Wastes Water.20th edition.* American Public Health Association. American Water Works Association. Water Environment Federation Washington D.C

Azubuike, A. (2016). Biocumulation and bioavailability of Benthic Organism (Oreochromis niloticus) in Wudil river, Nigeria. *Journal of Chemistry and Aquatic Science*s (IJCA), 2(1), 1-12. www.chemistryjournal.kupublication.com

Azubuike, A. (2019). Analysis of food and feeding habits clarias gariepinus in Tiger DAN Kano State, Nigeria. *Journal of Biotechnological Research*, 1(2), 64-75.

Bichi, A.H., Mukhtar,M.D. & Galtimari A.B (2002). Bacteriological analysis of drinking water in Kano Metropolis. *Journal of Life and Environmental Sciences 4(2):2*14-220.

Cheesbrough, M. (2000). *District laboratory practice in Tropical Countries.* Second Edition, Part 2. 160-190.

Cheesbrough, M. (2010). *District laboratory practice in Tropical Countries.* Cambridge University Press, U.K. 157-195.

Chioma, G., Nzeh & Augustine O. Udeze (2010). Lactobacillus delbruekii infection of *Clarias bidorsalis* cultured in tanks in Ilorin, Kwara State, Nigeria. *Agriculture and Biology Journal of North America.* http: //www. scihub. or g/AB JNA.

Edema, M.O., Osho, A.T., & Diala, C.I. (2008). Evaluation of microbial hazards associated with the processing of suya (a grilled meat product). *Scientific Research and Essay,* 3(12), 621-626.

Egbere, O.J., A, Kadir., T, Oyero., Steve, K., Odewumi, O., & Zakari, H. (2010). Bacteriological quality of catfish in Jos metropolis, Nigeria. *International Journal of Bioscience, 5* (2), 2010, 95-103.

Emikpe, B.O., Adebisi, T., & Adedeji, O.B. (2011). Bacterial load on skin and stomach of *Clarias gariepinus* and Oreochromis niloticus from Ibadan, Southeast Nigeria. Public health *implications. J.Microbiol.Biotech.Res.* l(l), 52-59.

EPA. (2003). *US Environmental Protection Agency Drinking Water Act.* EPA 816-F-03-016.

FAO/WHO (2005). *Joint FAO/WHO Activities on Risk Assessment of Microbiology Hazards in Foods.* Preliminary Document. Hazard Identification, Exposure Assessment and Hazards Characterization of *Vibrio spp* in sea Food. Availabble at:ftp://ftp.fao.org/es/esn/food/vibrio.pdf.

Food and Agricultural Organization (FAO) of the united nation. (2003). *Manuals of food quality control*-Microbiological Analysis. Pp A1-D30.

Food and Environmental Hygiene Department (FEHD) (2005). *Vibrio species in seafood .In: Risk Assessment Studies Report No 20 food and environmental hygiene deparment HKSAR Government. Guide* lines on the Alteration and disinfection facilities for fish tank water. http://www.fehd.gov.hk/pleasant-environment/library/fish- Tank/guide .html.

Harishi, R., Nishai, K.S., & Mohammed, A.A. (2003). Prevalence of opportunistic pathogens in Paddy cum Shrimp Farms Adjoining Vembanadu lake. Kerala, India, *Asia Fisheries Science*.16:607-625.

Kumar, J.S.S. (2004). Management of super intensive farming of African catfish. A publication by the Technical Services Division, *Animal Science illionois-Indiana Sea Grant Program Purdue University.*

Mukhtar, M. D; Oyeyi, T.I & Kawo, A.H. (2001). Enterobacteria infections in Journal of Kano State. *The Society of Experimental Biology* (NISEB)

NPC (National Population Commission) (2006). *State by state National Population Figures.* NPC Office Kano State, Nigeria.

Obiajaru, I.O.C., & Ogbulie, J.N. (2006). Bacteriological quality of some fishes and crabs from Rivers within Imo River Basin. *Journal of Aquatic Sciences, 21(1), doi:10.4314/jas.v2111.20054.*

Okonko, I.O et al. (2008). Comparative studies and microbial risk assessment of different Ready- To- Eat (RTE) frozen sea-foods processed in Ijora-Olopa, Lagos State, Nigeria. *African Journal of Biotech,* 7(16), 2898-2901.

Okonko, I.O., Ogun, A.A., Adejoye, O.D., Ogunjobi, A.A., Nkang, A.O. & Adebayo-Tayo, B.C. (2009). Hazards Analysis Critical Control Points (HACCP) and Microbiology qualities of sea-foods as affected by Handlers Hygiene in Ibadan and Lagos, Nigeria. *African Journal of Food Sciences,* 3(1), 035-050.

World Health Organization. (2007). Food safety and foodborne illness Geneva*. In* W.A. Wurts, & R.M. Durbow. (1992*). Interactions of pH, carbon dioxide, alkalinity and hardness in fishpond. Southern Regional Aquact. Centre Fact. Sheet* No 464:1-4

Yagoub, S. O. (2009). Isolation of *enterobacteriaceae* and *pseudomonas spp* from raw fish sold in fish markets in Khartoum State. *Journal of Bacteriology Research,* 1(7), 085-088.