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Isolation and Identification of Enterobacteriaceae in Catfish (*Clariasgariepinus* -Burchell 1822) gut from Selected Fish Farms in Ondo City, Ondo State

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Abstract:
Background: *Enterobacteriaceae are Gram-negative, short rods, non-sporulating, facultative anaerobes. This group of organisms include several that cause primary infections of the human gastrointestinal tract. Members of this family are major causes of opportunistic infection (including septicemia, pneumonia, meningitis and urinary tract infections). This study aimed at enumerating isolates belonging to Enterobacteriaceae present in Clariasgariepinus and their culturing water samples.*
Materials and Methods: *An unbiased 8 fish samples with an average weight of 425.5g were randomly collected from an earthen pond in 4 selected fish farm across the four cardinal of Ondo City metropolis. Water samples from the culturing ponds and their sources were collected in 250ml sterile water sampling bottles for microbial analysis. In-situ measurements of the physico-chemical parameters were done according to the APHA analytical method.Enterobacteriaceae were isolated from the small intestine and stomach of the fish samples and the isolates were subjected to various biochemical tests to aid in their identification.*
Results: *The highest mean total viable count was observed from the small intestine and stomach of fish samples collected from Ondo west (6.9 x 10^6 CFU/mL and 7.9 x 10^6 CFU/mL). Bacterial pathogens isolated included Escherichia coli, Pseudomonas aeroginosa, Salmonella spp., Shigella spp., Klebsiella spp., Citrobacterfreundii, Serratiamarcescenis, Enterobacter aerogenes and Proteus vulgaris.*
Conclusion: *This study revealed that fresh catfish obtained from earthen ponds in Ondo City, could be a source of food-borne bacterial pathogens, as the organisms isolated are of public health significance.*
Key Word: *Bacterial pathogens; food borne; public health; Ondo city; earthen ponds.*

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I. INTRODUCTION

According to some researchers, fish has been reported to be one of the affordable and widely available source of quality protein for human consumption[4] [1]. Fish and fish cultivation is an important source of income for fish farmers and also a source of employment in developing countries [3]. The African catfish (*Clariasgariepinus)* has been reported as an important fish for aquaculture and essential source of protein in Nigeria and aquaculture industries [5] due to its several favourable characteristics such as fast growth rate, high rate of fecundity, ability to tolerate a varying range of environmental conditions, ease of artificial breeding and good price [6].

Catfish pepper soup is one of the delicacies served in relaxation spots in Ondo City leading to an increase in the demand for fish in the city. There is therefore an increase in the number of fish farms to meet the high demand of catfishes in Ondo City. However, fish consumption can aid bacterial contamination due to their aquatic habitat which is easily polluted by various activities which results in the generation of waste pollution from homes, farmlands, industries and cultural habits such as the use of animal manure as pond fertilizer in fish farming. Contaminants from these practices can render such fish unfit for human consumption [7]as they take in large number of bacteria into their alimentary tract from food and water sediments which are capable of causing pathogenic diseases

Freshly caught fish microbial flora is largely a reflection of microbial quality of the waters from where they are harvested. The microorganism present in the environment enables it to enter the food chain through raw materials and is a major problem in convenience foods and mass catering [8]. All living organisms including fish coexist with a wide range of pathogenic and non- pathogenic microorganisms which possess complex defence

mechanisms which contribute to their survival. Fishes receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. The bacteria found in the digestive tract of fish are highly variable and are a reflection of their aqueous environment, especially the food choice of the individual fish [9]. It is considered that the quality of aquatic food depends on the quality of water where the fishes are caught and the sanitary conditions of the landing centre since microorganisms occur nearly everywhere in nature and occupy an important place in the life of human [10].

Members of the *Enterobacteriaceae* family are major causes of opportunistic infection (including septicemia, pneumonia, meningitis and urinary tract infections). *Enterobacteriaceae* are Gram-negative, short rods, non-sporulating, facultative anaerobes. *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Morganella*, *Providencia* and *Serratia* are some of the examples of *Enterobacteriaceae* that cause opportunistic ifections. This group of organisms includes several genera that cause primary infections of the human gastrointestinal tract. According to [11], most fish related food borne illness are traced to *Salmonella*, *Staphylococcus spp*., *Escherichia spp*., *Vibrio parahemolyticus*, *Clostridium perfringens*, *Clostridium botulinum* , and *Enteroviruses*.

The pathogenic bacteria associated with fish as classified by [12] are indigenous and non-indigenous bacteria; the non-indigenous bacteria contaminate the fish or their habitat one way or the other and examples include *Escherichia coli*, *Clostridium botulinum*, *Shigelladysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens* and *Salmonella*, while the indigenous bacteria pathogens are found living naturally in the fish's habitat for example, *Vibrio* species and *Aeromonas* species which are not harmful to fish but may be harmful to man on consumption [13]. Thus, the aim of this study was to assess and identify various *Enterobacteriaceae* present in *C. gariepinus* obtained from major fish farms in Ondo City. The findings of this research will add to knowledge on *Enterobacteriaceae*associated with freshly collected samples of *C. gariepinus* from the selected fish farms in Ondo City, Nigeria.

II. MATERIALS AND METHODS

Study Location: This study was carried out in Ondo city, South-Western Nigeria. The study was carried out on four randomly selected fish farms in Ondo East, Ondo West, Ondo North and Ondo South.

Sample Collection and Preparation: A total of eight (8) catfish samples were collected from earthen ponds. The fish samples were transported alive in sterile bags to the Department of Biological Sciences laboratory, University of Medical Sciences, Ondo, Nigeria, for analysis. The Total Length (TL), and standard length (SL) of the fish samples were measured using measuring board and recorded to the nearest meter, likewise the weight of the fish were taken using digital weighing balance and recorded to the nearest gram. The samples were dissected with a sterile dissecting set and 1g of the required organs were obtained into sterile sample bottles containing 5ml saline water. Sterile sample bottles were used for the collection of culturing water.

Physicochemical parameters of water samples: The pH, temperature, Total Dissolved Solids (TDS), conductivity of the culturing water samples were measured *in situ* using the TDS meterwhile the dissolved oxygen (DO) was measured using the dissolved oxygen meter. Nitrate (NO_2), Nitrite (NO_3), Alkalinity, and Total hardness were measured in situ colormetrically, using the (Insta-Test®) analytic kit.

Isolation of bacteria from fish organs

All the media used for microbiological analysis were prepared following the manufacturer's instructions. Sterilization of equipment and maintenance of sterility was carried out following the method described by [14]. The microbiological analysis of catfish organs was carried out as described by American Public Health Association [15].One gram of each organ (small intestine and stomach) preserved in 5 mL saline water was homogenized using an homogenizer (Searchtech™). One millilitre of each homogenate which served as the stock solution was serially diluted (10^{-1} to 10^{-5}). One millilitre from the higher dilutions (10^{-3} and 10^{-5}) were cultured using pour plate techniques. Total viable aerobic counts were observed and counted using the colony counter after incubation at for 24 hours (Swanson *et al.*, 2001). Specific coliform organisms were differentiated by IMViC tests. Eosin methylene blue (EMB) agar (Oxoid, England) was used for the cultivation of *Escherichia coli* and *Enterobacter aerogenes*. For the isolation and enumeration of *Salmonella and Shigella, Salmonella-Shigella*agar(SSA) was used. Each water sample from the ponds were serially diluted and the same procedure described above was used in isolating *Enterobacteriaceae* from it.

Identification of bacterial isolates

Pure isolates on agar plates were characterized by initial morphological examination of the distinct colonies. Various biochemical tests such as catalase test, coagulase test, citrate utilization test, oxidase test, triple sugar iron agar, urease test, sugar fermentation test, methyl red test and indole production test were conducted on the isolates

Statistical analysis

The data obtained were analyzed with SPSS version 25 (SPSS Inc., Chicago, IL). Using descriptive statistics and One-way Analysis of Variance (ANOVA) and presented as Tables and Mean ± Standard deviation (SD). The significance was determined at 95% level of confidence ($p \leq 0.05$).

III.RESULT

The mean total viable count (TVC) of isolates obtained from the small intestine of fish samples from Ondo west, east, north and south ranged from 3.4 x 10^6 to 6.9 x 10^6 CFU/mL. The mean total viable count (TVC) of isolates obtained from the stomach of fish samples collected from Ondo west, east, north and south ranged from 3.5 x 10^6 to 7.9 x 10^6 CFU/mL as shown in table no 1. There were no significant differences among the bacterial counts from the small intestine and stomach of fish samples from Ondo west, east, north and south (*P*> 0.05).

Table no 1: Mean Total Viable Count (TVC) of bacterial isolates obtained from fish samples from four fish farms.

LOCATION	TVC S.I (CFU/ml) x10^6	TVC S.H (CFU/ml) x10^6
ONDO EAST	6.2±5.1^b	6.0± 4.4^c
ONDO WEST	6.9±2.1^a	7.9± 1.8^a
ONDO NORTH	5.6± 2.8^c	6.3± 3.0^b
ONDO SOUTH	3.4± 4.1^d	3.5± 2.9^d

Note: Mean ± S.D with different superscript are significantly different at 5% level with a>b>c>d.
S.I: Small Intestine, S.H: Stomach, CFU; Colony Forming Unit, TVC; Total Viable Count

Percentage frequency of bacterial isolates

Data obtained showed that the highest bacterial species isolated from the fish samples in Ondo city were *E. coli and Salmonella* sp. with percentage occurrence of 22.2% while the lowest was *S. marcescens, P. vulgaris, Shigella*sp., *and E. aerogenes* with 5.5% as shown in figure no 1.

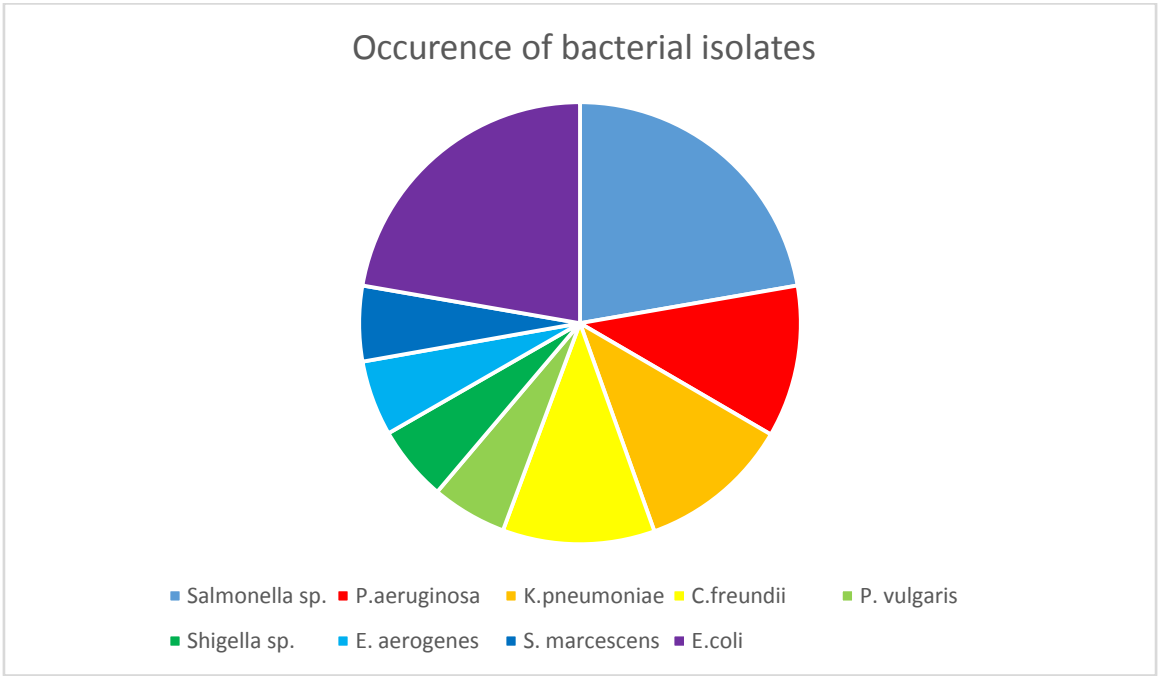

Figure no 1: Percentage occurrence of bacterial isolates from fish samples in Ondo city, Nigeria.

The mean temperatures of the ponds used in this study ranged from 26 ºC to 33.3ºC, mean pH ranged from 6.7 to 7.8, mean TDS ranged from 28 to 1482, mean DO ranged from 1.0 to 2.4, mean conductivity ranged from 0.1 to 2.0, mean total hardness ranged from 60 to 180 and mean alkalinity ranged from 80 to 240 as shown in table 2. It was observed that temperature and pH had no significant difference on the bacteria isolated ($p>0.05$) while the other parameters had high significance on the bacteria isolated ($p<0.05$),

Table no 2: Physicochemical parameters of pond water samples from four fish farms.

Parameters	Location			
	Ondo East	Ondo West	Ondo North	Ondo South
Temperature (^oC)	26.0 ± 1.4^b	30.0 ± 4.2^{ab}	29.0 ± 1.4^{ab}	33.3 ± 1.1^a
pH	6.7 ± 0.5^b	7.7 ± 0.3^a	7.2 ± 0.1^{ab}	7.8 ± 0.1^a
TDS	167.0 ± 2.8^d	820.0 ± 2.8^b	28.01 ± 2.8^c	1487.0 ± 2.8^a
D.O	$1.0\pm0.0.0^c$	2.4 ± 0.1^a	2.2 ± 0.1^a	1.4 ± 0.0^b
Conductivity	0.1 ± 0.0^d	1.0 ± 00^b	0.4 ± 0.0^c	2.0 ± 0.0^a
Total hardness	60.0 ± 0.0^a	120.0 ± 0.0^a	180.0 ± 0.0^a	180.0 ± 0.0^a
Alkalinity	80.0 ± 0.0^a	240.0 ± 0.0^a	180.0 ± 0.0^a	240.0 ± 0.0^a

Note: Mean ± S.D with different superscript are significantly different at 5% level with a>ab>b>c>d. Mean separation done by Duncan Multiple Range Test (DMRT).

Table no 3 shows the means of the weight and length of catfish samples used in this study. The mean of weights ranged from 206 g to 542.6 g. The mean of the lengths ranged from 24.2 cm to 42 cm. It was observed that the weight and length of the fish samples significantly affected the bacteria isolates obtained from the fish sample ($p<0.05$).

Table no 3: Weight and Length of Fish samples.

Parameters	Ondo East	Ondo West	Ondo North	Ondo South
Weight (g)	206.5 ± 36.2^b	209.3 ± 12.5^b	542.6 ± 154.2^a	243.1 ± 29.3^b
Length (cm)	30 ± 3.2^b	38.3 ± 4.6^a	42 ± 4.4^a	24.2 ± 5.7^b

Note: Mean ± S.D with different superscript are significantly different at 5% level with a>b. Mean separation done by Duncan Multiple Range Test (DMRT).

Table no 4: Morphological and biochemical characteristics of bacteria isolated from fish and water samples in selected fish farms in Ondo State, Nigeria.

KEY: R: Rod; (+): positive reaction; (-): negative reaction; A: Acid, no gas production; AG: Acid with gas production; G: No acid, gas production; (--): No acid, no gas production. Cat: Catalase, Ind: Indole, Mot: Motility, MR: Methyl red, VP: Vogues Proskaeur, Ure: Urease, Cit: Citrate, Glu: Glucose, Suc: Sucrose, Gal: Galactose, Man: Mannitol, Fru: Fructose, Mal: Maltose

IV.DISCUSSION

There are various culture systems used to rear fishes such as ponds (earthen or concrete), plastics and vats (wooden or fiber glass) [17]; [18]. Earthen and concrete ponds are the widely used culture systems in Nigeria with the earthen pond culture system as the most conventional method of aquaculture[18]. Fishes cultured with these types of culture system have been found to be contaminated by microorganisms which could be either pathogenic or opportunistic [18] and their presence in fish may constitute potential damage such as causing diseases to the consumers [20]. The findings from this research shows high bacterial load from fish samples collected from Ondo West. This may be as a result of different factors such as poor hygienic practices under which the fishes are cultured in the ponds, indiscriminate dumping of waste materials into ponds, runoffs, animal excreta, environmental wastes, free roaming of animals etc. All these can contribute to fecal contamination of the ponds [7]. Lack of technical/ skilled workers may also be a contributing factor [7]. Fish farm owners often wants to maximize profit and hence often employ unskilled pond keepers instead of trained aquaculture personnel [7].

This study was carried out during the dry season. In the dry season, there is little or no supply of water from the sources (rivers, streams, dams etc), making efficient drainage practices almost impossible. The farmers attested to poor draining of the ponds, a situation that favours bacteria reproduction and development in their host bodies. Human entry into ponds, use of dirty equipment (eg hand nets and cast nets) when cropping, feeding fish with bare hands all contribute to the occurrence of the bacteria species isolated from the tissues of *C. gariepinus*[7].

Earthen ponds absorb fish waste and fish feed wastes into the soil leading to an increase in microbial load of the pond. Accumulation of all these wastes will lead to an increase in the microbial load and hence a decrease in the dissolved oxygen available for use and this affects the quality of aquatic life. Bacteria is reported

to be abundant in the habitat where fish live and hence impossible to avoid them from being a component of their diet [21]. Life in dam and rivers will be lower than in the ponds because the pond has more organisms as a result of the accumulation of wastes, feeds etc as Total Dissolved Solids.

According to [22], physicochemical parameters such as alkalinity, dissolved oxygen, total hardness pH and temperature are the most common water quality characteristics that influence fish health and growth. The dissolved oxygen (DO) in all the ponds studied was between 1.0 – 2.4 mg/l which is below 5 mg/l recommended standard for fish culture [23]. Low levels of DO can put undue stress on fish and are most times linked to fish kill incidents[24]. However, it has been reported that the African cat fish (*Clariasgariepinus*) can survive in DO levels between 0.0-0.3mg/l because they are obligate air breathers. The pH of the fish pond water observed in this study falls within the standard range of 6.5- 8.0 [22].

All pH values recorded were within the standard range of 6.5-9.0 which is suitable for aquaculture. Although most fish can tolerate pH as low as 5.0, measurement of pH can help to determine if the water is a suitable environment for fish farming. The pH recorded in this study was similar to that of Njoku*et al*., 2015. The temperature observed from this study ranged from 26-33℃ and was within the limit that supports fish productivity. Temperature is a major factor that affects the ecosystem for aquaculture, as it also affects the organisms which can survive in the water bodies [18].

The *Enterobacteriaceae* isolated from the four ponds were majorly *Escherichia coli*, *Salmonella* sp., *Shigella*sp., *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. These organisms were also observed to be present on the small intestine and stomach of *C. gariepinus* from the four ponds.

It has been posited that dramatic changes in water quality result in poor health and low resistance of fish as a consequence, pathogenic threats are increased [22]. Infections, fish diseases, and deaths are all possible outcomes of dramatic changes in water quality. The physicochemical properties of the earthen fish ponds were found to be within the appropriate range, suggesting that the water quality was satisfactory and that it most likely may not affect the fish health.

Although the *Enterobacteriaceae* found has not been established as a cause of mortality to the fishes (probably due to the strong host defense response), the species however could become opportunistic and pathogenic which could result in fish diseases (Danba, *et al*., 2015). They are organisms of public health significance and could be involved in the transmission of diseases to human beings as fish and fish products have been reported as vehicles of food-borne bacterial infections in humans [25]; [7].This agrees with the study of [26]; [27] and [7].

The presence of *Pseudomonas* sp. in catfish could be of public health concern as it 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 [28]. *Enterococcus molodoratus* is a causative agent of dental infection and scarlet fever and has been implicated in human infections like pharyngitis, scarlet fever and pneumonia [21]. The isolation of enteric organisms such as *E. coli* is particularly useful as an indicator of faecal contamination [29]. The presence of *E. coli* may be due to the presence of faecal pollution caused by human and other environmental wastes in the water bodies from which the *C. gariepinus* was obtained, similar observation was also made by [30] and this could indicate possible presence of causative agents of gastrointestinal diseases [31]. The presence of *Pseudomonas* and *Proteus* species have been implicated in food poisoning [32]. The bacteria isolated from these major ponds are in agreement with the report of [18] who worked on the microbiological and physicochemical profiles of fish ponds.

V. CONCLUSION

This study revealed contamination of the major ponds with *Enterobacteriaceae*which are pathogenic and could affect fish cultivation, since the microbial quality of any pond water is a reflection of the microbial flora of the fish itself. Some of the members found in this study, such as *Salmonella* and *Shigella*are known fish/human pathogens and could lower yield, cause diseases and even endanger the consumers if under-processed.

Study on the antimicrobial resistance of *Enterobacteriaceae* is needed to be able to establish the medical problems posed by the ingestion of these organisms. The detection of enteric bacteria including potential pathogens in the ponds and fish samples suggests that strict hygiene habits should be practiced during handling, feeding and maintenance of fish. The general public should be enlightened on reasons why they need to ensure proper cooking prior to consumption, which is to prevent the transfer of potentially pathogenic bacteria to humans. Detection of *Enterobacteriaceae* can help to implement appropriate fish management practices and prevention of transmission of potential pathogens which is an important part of the food safety plan.

References

[1]. R. A. Castillo-Mercado, A. Bucio-Galindo, R. M. Salinas-Hernández, E. M. Aranda-Ibáñez, F. Izquierdo-Reyes, and J. A. Ramos-Juárez, "Milk quality of dual-purpose cows supplemented with biological fish silage (Pterygoplichthys sp.) as a protein source," *Rev. Colomb. Ciencias Pecu.*, vol. 33, no. 4, 2020, doi: 10.17533/udea.rccp.v33n4a05.

[2]. S. N. Musyoka, D. M. Liti, E. Ogello, and H. Waidbacher, "Utilization of the earthworm, Eisenia fetida (Savigny, 1826) as an alternative protein source in fish feeds processing: A review," *Aquaculture Research*, vol. 50, no. 9. 2019, doi: 10.1111/are.14091.

[3]. M. Madende and M. Hayes, "Fish by-product use as biostimulants: An overview of the current state of the art, including relevant legislation and regulations within the EU and USA," *Molecules*, vol. 25, no. 5, 2020, doi: 10.3390/molecules25051122.

[4]. B. Mohanty *et al.*, "Amino Acid Compositions of 27 Food Fishes and Their Importance in Clinical Nutrition," *J. Amino Acids*, vol. 2014, 2014, doi: 10.1155/2014/269797.

[5]. A. B. Dauda, I. Natrah, M. Karim, M. S. Kamarudin, and A. u H. Bichi, "African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects," *Fish. Aquac. J.*, vol. 09, no. 01, 2018, doi: 10.4172/2150-3508.1000237.

[6]. V. O. Eyo, A. P. Ekanem, and U. U. Jimmy, "A COMPARATIVE STUDY OF THE GONADO-SOMATIC INDEX (GSI) AND GONAD GROSS MORPHOLOGY OF AFRICAN CATFISH (Clarias gariepinus) FED UNICAL AQUA FEED AND COPPENS COMMERCIAL FEED Study area Formulation , composition and preparation of Unical Aqua feed," pp. 63–69, 2014.

[7]. E. Danba., "Occurrence of pathogenic bacteria associated with *Clarias gariepinus* in selected fish farms of Kumbotso local governement area of Kano state, Nigeria," *Bayero J. Pure Appl. Sci.*, vol. 7, no. 2, p. 145, 2015, doi: 10.4314/bajopas.v7i2.25.

[8]. FAO; WHO, "Food and Agriculture Organization Assuring Food Safety and Quality :," *Food Nutr. Pap.*, vol. 76, 2003, . Available: ftp://ftp.fao.org/docrep/fao/006/y8705e/y8705e00.pdf.

[9]. Z. Zhang, D. Li, W. Xu, R. Tang, and L. Li, "Microbiome of Co-cultured Fish Exhibits Host Selection and Niche Differentiation at the Organ Scale," *Front. Microbiol.*, vol. 10, no. November, pp. 1–11, 2019, doi: 10.3389/fmicb.2019.02576.

[10]. A. S. Al Sheraa, "Microbial Quality of three Imported Fresh Locally Produced Marine Fishes in Al-Faw City, Basrah, Iraq," *J. Aquac. Res. Dev.*, vol. 09, no. 04, pp. 9–11, 2018, doi: 10.4172/2155-9546.1000531.

[11]. S. Osman, Y. Ahmed, and S. O. Yagoub, "Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state," *J. Bacteriol. Res.*, vol. 1, no. 7, pp. 85–088, 2009, [Online]. Available: http://www.academicjournals.org/jbr.

[12]. J. E. Kvenberg, "Nonindigenous Bacterial Pathogens," *Microbiol. Mar. Food Prod.*, pp. 267–284, 1991, doi: 10.1007/978-1-4615-3926-1_10.

[13]. B. A. Saka, O. K. Adeyemo, and A. O. Odeseye, "Multiple antibiotic resistance indices of *Aeromonas hydrophila* isolates of muscle of catfish (*Clarias gariepinus*, Burchell 1822) from selected markets in Ibadan, Nigeria," *African J. Clin. Exp. Microbiol.*, vol. 18, no. 2, p. 73, 2017, doi: 10.4314/ajcem.v18i2.3.

[14]. I. J. Sawyer, "Methods of sterilisation.," *Nurs. J. India*, vol. 68, no. 7, p. 247, 1972.

[15]. 1992 APHA, "APHA Method 9221: Standard Methods for the Examination of Water and Wastewater," vol. 552.

[16]. K. M. SwansonJ., R. Petran L., and J. H. Hanlin, "Culture methods for enumeration of microorganisms. Compendium of methods for the microbiological examination of foods," *Am. Public Heal. Assoc. Washington, DC*, vol. 4th editio, pp. 53–62, 2001.

[17]. O. J. Olaoye, D. A. Adegbite, E. O. Oluwalana, I. O. Vaughan, C. O. Odebiyi, and A. P. Adediji, "Usporedba procjene ekonomske koristi zemljanih bazena ribnjaka i betonskih spremnika u akvakulturnom poduzetništvu države Oyo u Nigeriji," *Ribar. Croat. J. Fish.*, vol. 72, no. 3, pp. 107–117, 2014, doi: 10.14798/72.3.708.

[18]. F. Length, "An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria," vol. 9, no. 3, pp. 155–162, 2015, doi: 10.5897/AJFS2014.1208.

[19]. O. A. Davies, E. Ansa, and P. Harcourt, "COMPARATIVE ASSESSMENT OF WATER QUALITY PARAMETERS OF FRESHWATER TIDAL EARTHEN PONDS AND STAGNANT CONCRETE TANKS FOR FISH PRODUCTION IN PORT HARCOURT , NIGERIA Plate 1 ARAC tidal earthen ponds," vol. 1, no. 1, pp. 34–37.

[20]. M. T. Rahman *et al.*, "Zoonotic diseases: Etiology, impact, and control," *Microorganisms*, vol. 8, no. 9, pp. 1–34, 2020, doi: 10.3390/microorganisms8091405.

[21]. W. A. Jimoh, M. O. Oladele-bukola, A. A. Yusuff, and F. A. Azeez, "Sokoto Journal of Veterinary Sciences Microbial flora of the gastro-intestinal tract of Clarias gariepinus caught from river Dandaru Ibadan , Nigeria," vol. 12, no. 2, pp. 19–24, 2014.

[22]. N. Torimiro, P. T. Bebe, E. F. Ogundipe, M. D. Esan, and I. A. Aduwo, "The Bacteriology and Physico-chemical Analysis of Freshwater Fish Ponds," *Int. Res. J. Microbiol.*, vol. 5, no. 3, pp. 2141–5463, 2014, [Online]. Available: http:/dx.doi.org/10.14303/irjm.2014.014%5Cnhttp://www.interesjournals.org/IRJM.

[23]. C. E. Boyd, D. Ph, and T. Hanson, "production Concentrations In Pond Aquaculture," no. February, pp. 40–41, 2010.

[24]. Golam Kibria, "Global fish kills: Causes & Consequences," *Sydneybashi-Bangla*, no. November, pp. 6–7, 2011, doi: 10.13140/RG.2.1.1422.8965.

[25]. B. Hjeltnes, A. Lillehaug, and M. H. G. Berntssen, "Food safety hazards that occur during the production stage : challenges for fish farming and," no. May 2014, 2006.

[26]. A. O. Ajayi, "Bacteriological Study of Catfish , Claria gariepinus , from Fish Pond Sources in Akungba-Akoko Community , Nigeria," vol. 2, no. 1, pp. 1–9, 2012.

[27]. B. O. Emikpe, T. Adebisi, and O. B. Adedeji, "Bacteria load on the skin and stomach of Clarias Gariepinus and Oreochromis Niloticus from Ibadan , South West Nigeria : Public health implications," vol. 1, no. 1, pp. 52–59, 2011.

[28]. O. T. Osuntokun and A. A. Muniru, "Bacteriological Assessment of African Catfish (Clarias gariepinus) Isolated from Earthen and Concrete Fish Pond," vol. 6, no. 1, pp. 17–34, 2020, doi: 10.9734/AJBGMB/2020/v6i130143.

[29]. C. Rodrigues, "Assessment of the microbiological quality of recreational waters : indicators and methods," 2017, doi: 10.1007/s41207-017-0035-8.

[30]. J. Jang, H. G. Hur, M. J. Sadowsky, M. N. Byappanahalli, T. Yan, and S. Ishii, "Environmental Escherichia coli: ecology and public health implications—a review," *Journal of Applied Microbiology*, vol. 123, no. 3. pp. 570–581, 2017, doi: 10.1111/jam.13468.

[31]. J. A. Ampofo and G. C. Clerk, "Diversity of Bacteria Contaminants in Tissues of Fish Cultured in Organic Waste-Fertilized Ponds : Health Implications," pp. 142–146, 2010.

[32]. T. A. Oni, V. F. Olaleye, B. O. Omafuvbe, "Preliminary studies on associated bacteria and fungal load of artificially cultured," vol. 15, no. 1, pp. 9–16, 2013.

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