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# Bacterial flora of *Clarias gariepinus* from some selected fish ponds in Port Harcourt

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*ORIGINAL RESEARCH ARTICLE*

## ABSTRACT

*Clarias gariepinus* is a popular fish species for aquaculture practices. Aquaculture has gained recent popularity due to the emphasis on fish which is considered as a source of animal protein. Currently, the bacterial infection in *C. gariepinus* is a major concern. The bacterial flora of *C. gariepinus* sourced from two (2) ponds in Port Harcourt was studied. Using the spread plate technique, the total viable bacterial load in the water, skin, gills and intestine was investigated. The mean total viable aerobic bacterial count was 0.3350±0.021 x $10^8$ CFU/mL, 0.170±0.014 x $10^8$ CFU/mg, 0.025±0.0071 x $10^8$ CFU/mg and 1.71±0.071 x $10^8$ CFU/mg, respectively for the water, skin, gills and intestine for samples obtained from pond 1. Similarly, pond 2 had 0.0075±0.00071 x $10^8$ CFU/mL, 0.3500±0.014 x $10^8$ CFU/mg, 1.59±0.0071 x $10^8$ CFU/mg and 0.45±0.071 x $10^8$ CFU/mg for the water, skin, gills and intestine, respectively. The isolated bacteria showed a wide diversity. Nine different species of bacteria were identified. They include *Bacillus* spp. (18.6%), *Staphylococcus* spp. (17.0%), *Streptococcus* spp. (17.0%), *Proteus* spp. (11.9%), *Escherichia coli* (10.1%), *Salmonella* spp. (8.5%), *Serratia* spp. (5.1%), *Pseudomonas* spp. (5.1%) and *Enterobacter* spp. (5.1%). These results indicate the presence of heavy infection in the examined fish, although they appeared physiologically healthy. The presence of these organisms could constitute a public health risk and calls for adequate preventive measures.

## KEYWORDS

aquaculture; bacterial flora; *Clarias gariepinus*; infection; public health

## 1. INTRODUCTION

Bacteria are ubiquitous and have successfully colonized every known habitat in the biosphere (Wiley et al., 2008; Postolec et al., 2012). The ability to colonize multiple habitats has been attributed to the diversity in the physiology and phylogeny of this group of organisms (Barberán et al., 2014). Bacteria can be broadly classified as Gram positive and Gram negative organisms on the basis of their cell wall structure (Wiley et al., 2008; Silhavy et al., 2010). On the basis of pathogenicity, they may be further classified into non-pathogenic and pathogenic flora (Fuchs et al., 2012). Studies by various researchers (Danba et al., 2014; Efuntoye et al., 2012; Ikpi and Offem, 2011) have pointed out that the Gram negative bacteria is the most common bacteria present in fish. These studies further reported that Gram positive and acid fast bacteria also infect fish.

Fish has recently become a preferred source of animal protein (Danba et al., 2014). Fish supplies up to 40% of the total animal protein required for humans. It is known to contain high protein content and, other nitrogenous constituents but low in cholesterol (Jay, 1996). However, the importance of fish products is not limited to nutrition but also considered as a good item for international trade, thus earning foreign exchange (Yagoub, 2009; Adebayo et al., 2012). The shift in interest towards fish has led to cultivation of fish through aquaculture practices. Like

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all ventures undertaken by man, profit is of essence in aquaculture. Clarias gariepinus is a preferred fish species in aquaculture due to its hardy nature, ease of larval production in captivity and good market price (Herald, 1971; Efuntoye et al., 2012). Similarly, Ikpi and Offem (2011) suggested that *C. gariepinus* is good for aquaculture based on their palatability, fecundity, disease resistance and high growth rate. Based on its great economic value, *C. gariepinus* is becoming an endangered species (Ikpi and Offem, 2011). Thus there is a need to encourage preservation through aquaculture (Fagbenro et al., 1993).

The successful cultivation of fish through aquaculture practice in Nigeria is challenged by bacterial infections. About 50% of fish from aquaculture perish due to bacterial infections (Ikuromo, 1981). Other studies showed that bacterial load in healthy fish range from $10^2$ to $10^7$ CFU/cm$^2$ in the skin, $10^3$ CFU/g for the gill and $10^7$ CFU/g in the intestines. The interaction between fish pathogen and the prevailing environment is largely responsible for fish infections (Ikpi and Offem, 2011). Other studies have implicated raw materials, personnel and processing channels for fish contamination (Danba et al., 2014). The implication of this heavy infection is enormous. The fish may serve as carriers of these infections, hosting bacteria that may cause human infections (Danba et al., 2014). Infections resulting from fish are rare but could result in fatalities, especially if the fish consumed is not properly cooked (Adebayo et al., 2012; 2012; Yagoub et al., 2009; Danba et al., 2014).

Although food poisoning, death or mass mortality in fish ponds is not yet widespread in Nigeria, there is need to study the bacterial flora and microbiological quality of *C. gariepinus* in Nigeria. This study therefore aims at enumerating and isolating the total aerobic bacteria associated with *C. gariepinus* harvested from two fish ponds in Port Harcourt and highlight the significance of the bacteria in the environment.

## 2. MATERIALS AND METHODS

### *2.1. Test Organism*

Ten adult *Clarias gariepinus* (mean weight 175.25 ± 4.59 g and 356.2 ± 8.77 g) each from pond 1 and 2 respectively was sampled in Port Harcourt. The fish were pitted and dissected to obtain its parts for bacterial enumeration, isolation and identification.

### *2.2. Enumeration and isolation of heterotrophic bacteria*

Bacterial load of the fish as well as its water habitat was studied. Various fish parts were obtained for analysis. Skin scrapping was aseptically collected and 1.0 g was suspended in 9.0 mL of sterile peptone water and homogenised to obtain 1:10 dilution (Sowunmi et al., 2008). Fish was dissected to extract the internal parts. Ten grams of the gill was suspended in 90 mL of sterile peptone water. Similarly, 1 g of intestine, chopped into tiny pieces, was suspended in 9 mL of peptone water. Both set up were mixed vigorously. One mL of the pond water was added to 9 mL of sterile peptone water. All set up were then diluted to $10^{-5}$ as described by Wiley et al. (2008). Then 0.1 mL of the appropriate dilution were pipetted from the $10^{-3}$ to $10^{-5}$ dilutions and plated using the spread plate method (Apha, 1976; Horsely, 1977). Plates were incubated at 30 $^{o}$C for 24 h. All treatments were done in triplicates. Colonies between 30 and 300 were counted and recorded. Representative colonies were then described and subcultured on sterile nutrient agar plates for identification.

### *2.3. Identification of bacterial isolates*

Identification of bacterial isolates was based on the methods of Buchanan and Gibbons (1994), Cowan (1974), and Cruickshank et al., (1984). These tests included Gram's stain, Catalase test, Oxidase test, Coagulase test, Motility test, Methyl-red test, Voges – Proskaur test as well as Sugar Fermentation test.

### *2.4. Data analysis*

Statistical analysis was carried out on the data which was obtained during the study. Analysis of variance and Student Newman Keul's (S-N-K) test were used to test for significance and mean separation respectively. This was done using a computer-based programme - SPSS version 17.

## 3. RESULTS AND DISCUSSION

Although all fish samples used in this study appeared physiologically healthy, they were heavily infested with various bacteria species. Bacterial infestation is encouraged by certain predisposing factors (Danba et al., 2014). Results of the bacterial populations of the pond water and the tissues of *C. gariepinus* are

**Table 1.** Analysis on the variation of mean – standard deviation of total heterotrophic bacterial counts of the different tissues (x$10^8$ CFU/g) and water (x$10^8$ CFU/g) from the ponds.

| Ponds | Intestine | Gills | Skin | Water |
|---|---|---|---|---|
| **Pond 1** | 1.71±0.071 | 0.025±0.0071 | 0.1700±0.014 | 0.3350±0.021 |
| **Pond 2** | 0.45±0.071 | 1.59±0.0071 | 0.3500±0.014 | 0.0075±0.00071 |

**Table 2.** ANOVA showing the level of significance (p≥0.05) of heterotrophic bacteria in different tissues and water at the examined ponds

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| **Intestine * Ponds** | Between groups (combined) | 1.588 | 1 | 1.588 | 317.520 | .003 |
| | Within groups | .010 | 2 | .005 | | |
| | Total | 1.598 | 3 | | | |
| **Gills * Ponds** | Between groups (combined) | 2.434 | 1 | 2.434 | 48672.000 | .000 |
| | Within groups | .000 | 2 | .000 | | |
| | Total | 2.434 | 3 | | | |
| **Skin * Ponds** | Between groups (combined) | .032 | 1 | .032 | 162.000 | .006 |
| | Within groups | .000 | 2 | .000 | | |
| | Total | .033 | 3 | | | |
| **Water * Ponds** | Between groups (combined) | .107 | 1 | .107 | 476.165 | .002 |
| | Within groups | .000 | 2 | .000 | | |
| | Total | .108 | 3 | | | |

presented in Table 1. Total viable bacterial count in the different parts studied was highest in the intestine (1.71±0.071 x $10^8$ CFU/g) and gills (1.59±0.0071 x $10^8$ CFU/g) in pond 1 and 2, respectively (Table 1). Test of significance showed that there was a significant difference (p≥0.05) in all the samples analyzed at the ponds (Table 2). The high levels of the bacterial load in the gills could be due to the bacteria being trapped in the gills during respiration via the gills. The intestine is also known to be a normal habitat for most bacteria hence more bacteria were present in the intestine. These results also agree with other researchers who recorded high levels of bacteria in the gills and intestine of *C. gariepinus* exposed to various effluents (Sowunmi et. al., 2008; Akani and Obire, 2014). These levels, however, defile the set microbial food safety standard of 1 to 100 CFU/g (Nester et al., 2004).

A visit to both ponds revealed that the water in pond 1 was changed less frequently than pond 2. This suggested better pond management in pond 2 as compared to pond 1. While water in pond 2 appeared clear, the water in pond 1 was more turbid. This may be why the morphometry of the fish in pond 2 appeared healthier in terms of weight measured. Akani and Obire (2014) had recorded that a reduction in dissolved oxygen as well as high turbidity affected the weight of *C. gariepinus* which was exposed to oilfield wastewater. Several studies also suggested a strong relationship between the prevailing conditions of the water environment and bacterial load of fish (Efuntoye et al., 2012; Ikpi and Offem, 2011; Danba et al., 2014). All studies indicated that poor aquaculture practices could be responsible for the various levels of bacterial load as observed in this study.

The study revealed a high diversity of bacteria present in *C. gariepinus*. There were 59 isolates, representing 9 different bacterial species in the parts studied (Figure 1). *Bacillus* spp. (18.6 %) *Streptococcus* spp. (17.0%) and *Staphylococcus* spp. (17.0%) were most prevalent bacterial species. Conversely, *Enterobacter* spp., *Pseudomonas* spp. and *Serratia* spp. (5.1% each) were least. The presence of these bacteria suggests availability of various necessary growth factors in the studied ponds as suggested by the previous studies (Ikpi and Offem 2011; Danba et al., 2014). In addition, the presence of certain normal flora of humans such as *Staphylococcus* and *Streptococcus* suggest poor aquaculture practices (Danba et al., 2014; Ikpi and Offem 2011). Danba et al. (2014) implicated poor practices, such as handling, for fish contamination. Enteric bacteria such as *E. coli* have been used as indicators of the microbiological quality of food and water (Tortorello, 2003). The presence of these indicator organisms calls for more aseptic practices. This condition resulted as the pond operators used animal manure for their aquaculture.

This is very common in Nigeria, even though it is not encouraged due to the danger to public health (Efuntoye et al., 2012). Certain strains of *E. coli* have also been associated with fetal infections (Temmelli, 2002; Manna et al., 2008; Efuntoye et al., 2012). This necessitates a caution in aquaculture practices as a result of this possible public health issue.

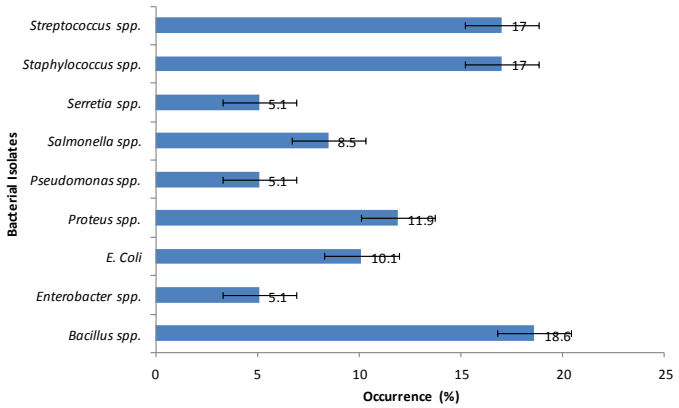


**Figure 1.** Diversity and incidence of bacteria in *C. gariepinus* in the analyzed samples.

Isolates from this study as well as previous studies have been indicated as pathogens of human as well as fish (Ikpi and Offem, 2011; Efuntoye et al., 2012). *Salmonella* spp. *Staphylococcus* spp. are known to cause severe diseases in man while *Pseudomanas* spp. and *Proteus* spp. have been identified as responsible for some fatal diseases in fish as well as zoonotic to man (Raghavan, 2003; Babu, 2000). *Bacillus* and *Staphylococcus* spp. are major pathogens causing food borne diseases in the United States (Bennett et al., 2013).

## 4. CONCLUSIONS

This study demonstrated several pathogenic bacteria associated with *Clarias gariepinus*. Although these fish appeared healthy at the time of this study, the organisms isolated could pose a public health hazard. The fish studied were contaminated since bacterial load defiled normal threshold allowed for food. Most pathogenic organisms present could have been introduced by the practices adopted in these ponds. There is a need to review these practices in order to reduce the health hazards. The need to employ the Hazard Analysis Critical Control Points (HACCP) in the aquaculture is necessary to manage and reduce the levels of contaminants in our fish ponds. Adequate cooking methods are widely employed in Nigeria prior to fish consumption. This is likely the reason why there is no wide mortality and morbidity from this highly infected protein source. These safe preparation methods are encouraged. This could prevent public health issues due to the consumption of *C. gariepinus*.

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## REFERENCES

Adebayo, E.A., Majolagbe, O.N., Ola, I.O., and Ogundiran, M.A. (2012) Antibiotic resistance pattern of isolated bacteria from salads. Journal of Research in Biology, 2, 136-142.

Akani, N.P. and Obire O. (2014) Bacterial populations of *Clarias gariepinus* (Burchell 1822) exposed to an oilfield wastewater in Rivers State, Nigeria. Asian Journal of Biological Sciences 7, 208 - 216.

APHA. (1976) Standard Methods for the Examination of Water and Wastewater, 14th Edition, APHA American Public Health Association.

Babu, P.S. (2000) Ichyozoonoses. Fish Farmer International, 14, 14-17.

Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H. and Fierer, N. (2014) Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. Ecology letters, 17, 794-802.

Bennett, S.D., Walsh, K.A. and Gould, L.H. (2013) Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* in United States, 1998–2008. Clinical Infectious Diseases, 57, 425-433.

Buchanan, R.E. and Gibbons, N.E. (1994) Bergey's Manual of Determinative Bacteriology, 9th edition. The Williams and Wilkins Company, Baltimore.

Cowan, S.T. (1974) Manual for Identification of Medical Bacteria (2nd edition). Cambridge University Press, Cambridge, 17 – 174.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain. R.H.A. (1984) Medical Microbiology. Vol. 2, 13th Edition. Churchill Livingstone, New York.

Danba, E.P., Bichi, A.H., Ishaku, S., Ahmad, M.K., Buba, U., Bingari, M.S., Barau, B.W. and Fidelis, U.F. (2014) Occurrence of pathogenic bacteria associated with *Clarias gariepinus* in selected fish farms of Kumbotso local governement area of Kano state, Nigeria. Bayero Journal of Pure and Applied Sciences, 7, 145-149.

Efuntoye, M.O., Omotosho, O.O. and Ashidi, J.S. (2012) Prevalence of methicillin-resistant *Staphylococcus aureus* and coagulase negative *Staphylococci* among male students in a private tertiary institution and their enteritoxin-producing potential. Asian Journal of Pharmaceutical Health Care and Sciences, 2, 231-234.

Fagbenro, O.A., Balogun, B., Ibironke, N. and Fasina, F. (1993) Nutritional values of some amphibian in diets for *Clarias gariepinus* (Burchell, 1822) (Siluroformes: Clariidae). Journal of Aquaculture in the Tropics, 8, 95-101.

Fuchs, T. M., Eisenreich, W., Heesemann, J., and Goebel, W.

(2012) Metabolic adaptation of human pathogenic and related nonpathogenic bacteria to extra-and intracellular habitats. FEMS Microbiology Reviews, 36, 435-462.

Herald, S.E. (1971) Living Fishes of the World, 1st Edition. Doubleday and Company Inc., New York. pp. 126.

Horsley, R.W. (1977) A review of the bacterial flora of teleosts and elasmobranchs, including methods for its analysis. Journal of Fish Biology, 10, 529-553.

Ikpi, G. and Offem, B. (2011) Bacterial infection of mudfish *Clarias gariepinus* (Siluriformes Clariidae) fingerlings in tropical nursery ponds. Revista de Biología Tropical, 59, 751-759.

Ikuromo, E.I. (1981) Identification of Parasites of *Clarias gariepinus* in Rivers State. Unpublished HND Project, Rivers State University of Science and Technology, Port Harcourt.

Jay, J.M. (1996) Modern Food Microbiology 4th edition, CBS Publishers and Distributors, India.

Manna, P., Sinha, M. and Sil, P.C. (2008) Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Archives of Toxicology, 82, 137-149.

Nester, E.W., Anderson, D.G., Roberts Jr, C.E., Pearsall, N.N., Nester, M.T. and Hurley, D. (2004) The Molecules of life. Microbiology: A Human perspective, 4th edition, McGraw Hill, Companies. Inc, New York, 311.

Postollec, F., Mathot, A.G., Bernard, M., Divanac'h, M.L., Pavan, S. and Sohier, D. (2012) Tracking spore-forming bacteria in food: from natural biodiversity to selection by processes. International Journal of Food Microbiology, 158, 1-8.

Raghavan, R.P. (2003) Incidence of human pathogenic bacteria in shrimp feeds-A study from India. Naga, World Fish Center Quarterly, 26, 22-24.

Silhavy, T.J., Kahne, D. and Walker, S. (2010) The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology, 2, a000414.

Sowunmi, A.A., Okunubi, M.A., and Efuntoye, M.O. (2008) Occurrence of bacteria in gills and buccal cavity of *Clarias gariepinus* (Burchell, 1822) and *Tilapia zillii* (Gervais) from Lekki lagoon, Southwest Nigeria. World Journal of Biological Resources, 1, 14-17.

Temelli, S. (2002) Food poisoning agent *Escherichia coli* O157: H7 and its importance. Journal of the Faculty of Veterinary Medicine, 21, 133-138.

Tortorello, M.L. ( 2003) Indicator organisms for safety and quality-uses and methods for detection: minireview. Journal of AOAC International, 86, 1208-1217.

Wiley, J.M., Sherwood, L.M. and Woolverton, C.J. (2008) Prescott, Harley and Klein's Microbiology 7th edition, McGraw Hill Higher Education, New York, 1088.

Yagoub, S.O. (2009) Isolation of Enterobacteriaceae and *Pseudomonas* spp. from raw fish sold in fish market in Khartoum state. African Journal of Bacteriology Research, 1, 85-88.