

Assessment of Bacteria on *Clarias gariepinus* AND *Gymnarchus niloticus* From Delta Region (Ese Odo), Southwest Nigeria

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Abstract

Investigation of state and influence of environment on bacteria occurrences, load and prevalence on two fish species (*Clarias gariepinus* and *Gymnarchus niloticus*) of economic importance in Nigeria was carried out in Ese-Odo delta (aquatic) environment. A total of 46 fish samples comprising of 23 *Clarias gariepinus* and 23 *Gymnarchus niloticus* were collected from the Ese-Odo delta environment and assessed for bacteria occurrence, prevalence and load. Seventeen species of bacteria were recovered from *Clarias gariepinus* while five bacteria species recovered from *Gymnarchus niloticus*. Bacteria load on the body of *Clarias gariepinus* indicated $67.28 \pm 29.11 \times 10^3$ on skin, $30.14 \pm 29.16 \times 10^3$ on the intestine, $22.57 \pm 9.57 \times 10^3$ on the gill and $11.71 \pm 17.34 \times 10^2$ on the liver, while *Gymnarchus niloticus* was $60.29 \pm 25.65 \times 10^3$ on skin, $11.14 \pm 3.43 \times 10^3$ in intestine, $5.17 \pm 2.05 \times 10^3$ in the gills and in the liver $3.14 \pm 2.47 \times 10^2$. Prevalence of bacteria was highest on the skin. The bacteria species include: *Bacillus* sp, *Streptococcus* sp, *Spirillum* sp, *Pseudomonas* sp., *Escherichia coli*, *Vibrio* sp, *Proteus* sp, *Aerococcus* sp, *Lactobacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Fusobacterium* sp, *Citrobacter* sp, *bacteriodes* sp, *zoogloea* sp, *Campylobacter* sp, *Alcoligenes* sp, and *Xanthomonas* sp. *Staphylococcus* sp prevailed most with 33% in *G. niloticus* and 16% in *C. gariepinus*; and significant difference was realized in bacteria load and prevalence on *C. gariepinus* to *G. niloticus* fish collected from Ese-Odo delta environment.

Keywords: Bacteria, *Clarias gariepinus*, *Gymnarchus niloticus*, Esa-Odo, Environment, bacteria

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I. Introduction

Clarias gariepinus is specie of catfish of the family *Clariidae*, commonly called African mud cat fish. They are found throughout Africa and the Middle East, and are endemic to freshwater, lakes, rivers, swamps as well as induced environments (Wikipedia 2011). *Clarias gariepinus* is named after its locality in Gariep River, a habitat name for Orange River in south Africa (Teugels, 1986).

Gymnarchus niloticus (Cuvier 1829) also known as trunk fish, and locally called Aba knife fish. It belongs to the family *Gymnarchidae* and it is the only living specie of this family (Ayoola *et al.*, 2010). They are classified under the order *Osteoglossidae* (the bony togued fishes). They are usually found in Africa, in River Nile, Niger, Volta and kanji dam.

Apart from the high perishability of fish, consumer safety is an issue to be considered because fish is a good medium for rapid bacteria multiplication particularly when processed under unsanitary conditions. Fish is processed mainly by smoke-drying in Nigeria, however, smoking may not commence immediately after capture as fresh fish is usually left at ambient temperatures where bacterial proliferation is encouraged. Shewan (1977) and Austin (2002) observed that microorganisms associated with freshly caught fish are principally a function of the environment where it is caught. According to Lima dos Santos (1978) tropical freshwater fish have a microbial flora comprising 54% gram negative and 43% gram positive bacteria; while the flora of tropical marine fish species are 60% gram negative and 37% gram positive.

Generally, microbial load increases on freshly caught fish where appropriate preservation techniques are not employed immediately after catch. As the natural defenses of fish break down as a result of death, available nutrients are used by microorganisms to sustain their life processes and support rapid multiplication. With an increase in bacterial flora and load, decomposition of the fish is rapid.

II. Materials and Methods

Live *Clarias gariepinus* and *Gymnarchus niloticus* fish species were obtained from Ilaje water, Igbokoda in Ilaje- Ese Odo Local Government of Ondo-state in the South West of Nigeria, and transported 25

liters plastic containers to laboratory. Samples were separated into different sexes and morphometric measurement on standard length (cm) and weight (g) were recorded.

One gram of skin, intestine, gill and liver samples were taken from the two fish species respectively for bacteria isolation using serial dilution method to 10^3 dilution; spread on petri dishes containing nutrient agar prepared at 121°C for 15 minutes; and incubated for 24 hours at 37°C . Colony forming counts of specimens from skin and stomach was determined using standard methods (Horsely, 1977, APHA, 1995). Each distinct colony was further sub-cultured on freshly prepared Nutrient Agar; and pure isolates obtained were stored on slants of Nutrient Agar in the refrigerator at 4°C . Identification of recovered bacterial was carried out using colonial, morphological and biochemical characteristics of colonies. Physical observation of surface colonies on nutrient agar medium was used to determine the colour, edge, elevation, surface, shape and arrangement of microorganisms. Morphological characteristics were studied under the oil lens immersion microscope after Gram-staining.

Biochemical tests carried out on the bacterial isolates were Catalase test, Coagulase test, Motility test, and Sugar Fermentation tests. One percent sugars such as glucose, sucrose, lactose, maltose, and others were used in a basal fermentative medium to determine the ability of the organisms to utilize the appropriate carbon sources signified by acid production or the change in colour of the medium and production of gas in Durham tube provided for the test. Recovered bacterial are counted to know most occurred and prevailed bacterial for health.

III. Results and Discussion

Morphometric measurement indicated mean weight (260.0 ± 48.64) g and mean standard length (29.71 ± 4.68) cm for *Clarias gariepinus* and mean weight (368.57 ± 34.84) g and mean standard length (28.71 ± 3.98) cm for *Gymnarchus niloticus* fish species.

Eighteen species of bacteria were recovered from the two fish species: *Clarias gariepinus* and *Gymnarchus niloticus*, seventeen species of which were recovered from *C. gariepinus*, while five species were recovered from *G. niloticus*. These bacterial include: *Bacillus spp*, *Streptococcus spp*, *Spirillum sp*, *Pseudomonas sp.*, *Escherichia coli*, *Vibro sp*, *Proteus sp*, *Aerococcus sp*, *Lactobacillus sp*, *Micrococcus sp*, *Staphylococcus sp*, *Fusobacterium sp*, *Citrobacter sp*, *Bacteriodes sp*, *zoogloea sp*, *Campylobacter sp*, *Alcoligenes sp*, and *Xanthomonas sp*. *Staphylococcus sp* had the highest occurrence (33%) in *G. niloticus* and 16% in *C. gariepinus*.

Bacteria load recovered within specie indicated *C. gariepinus* skin had highest bacterial load recovery ($67.28 \pm 29.11 \times 10^3$), $30.14 \pm 29.16 \times 10^3$ in intestine, $22.57 \pm 9.57 \times 10^3$ in gill and $11.71 \pm 17.34 \times 10^2$ in liver; while *G. niloticus* skin had highest bacteria load recovery ($60.29 \pm 25.65 \times 10^3$), $11.14 \pm 3.43 \times 10^3$ in intestine, $5.17 \pm 2.05 \times 10^3$ in gills and $3.14 \pm 2.47 \times 10^2$ in liver. And bacterial load between species indicated that *C. gariepinus* skin, intestine, gill and liver had higher bacterial load than the specimen skin, intestine, gill and liver of *G. niloticus*.

Within specie, *E. coli* had the highest percentage occurrence (100%) and in the gill of *G. niloticus*; and highest prevailing among species (Table 1 and 2). *Bacillus* and *Proteus* occurred most in liver of *C. gariepinus* while *G. niloticus* had no bacteria recovery from liver. Ten bacteria species were recovered from *C. gariepinus* skin with *Aeromonas* and *E. coli* occurring most, four bacteria were recovered from intestine with *Bacillus sp* (40%) occurring most; five bacterial from gill with *Staphylococcus* and *Spirillum* occurring most (28.6%) respectively; while *G. niloticus* had five bacterial occurrence on skin.

E. coli prevailed in the skin of *C. gariepinus*, *Bacillus sp* in intestine, *Staphylococcus sp* and *Spirillum sp* in gills, *Bacillus sp* and *Bacteriodes sp* in liver of *C. gariepinus*. *Staphylococcus sp* prevailed in skin of *G. niloticus*, *Streptococcus sp* and *Staphylococcus sp* in intestine, and *E. coli* in gills, while no bacteria recovery was made in liver.

E. coli is often used as an indicator for faecal contamination; however because of the ubiquitous nature of this organism in the tropics, this association is questionable. Some strains of *E. coli*, are capable of causing foodborne disease, ranging from mild enteritis to serious illness and death. These bacteria species may therefore be opportunistic. Association of bacteria with specific fish disease has not been successful in *C. gariepinus* as this fish species is regarded as a rather resistant fish (Huisman and Richter, 1997). Most strains of *E. coli* are harmless, and are occasionally responsible for product recall (Hudalt *et al.*, 2001). The harmless strain is part of the normal flora of the gut, and can benefit their host by producing Vitamin k2 and by preventing the establishment of pathogenic bacteria within the intestine (Reid *et al.*, 2001). *E. coli* is probably a normal flora of the fish (*Clarias gariepinus*). Other experimental study reveals that *Pseudomonas* and *Salmonella* species, caused peeling of the outermost skin and reduced appetite of the fishes (Udeze *et al.*, 2012).

Though there has not been much study on Ilaje river but different studies, Ekundayo 1997 on Lagos lagoon; Ajiwe *et al.*, (2000) on Ele River; isolated different bacterial species with potential for causing high proportion of deaths and ill health, in population dependent on the water bodies for water related resources. The

varieties of bacterial species were more from wild fishes as reported by other workers (Horsely 1973, Korie-Siakpere and Evbakhare, 1992, Sowumi *et al*, 2008) which reflected the prevailing conditions of water quality in different aquatic environments examined.

IV. Conclusion

The investigation on occurrence of bacterial flora on *Clarias gariepinus* and *Gymnarchus niloticus* indicated that microbial load prevailed on the skin, as it is prone to microbial contacts in water which is the common environment for micro-organisms and fish. High percentage of losses in fish production enterprise can be as a result of microbial infection and water quality management to reduce emergence of disease in fish so as to achieve financial and nutritional benefits in fish production. Hence, there is need for good fish culture. Occurrence of bacteria may be due to the pollution, therefore local communities should be educated on the effect of pollution on the water and the detrimental effect to fish health; and in the long run affect human's health.

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Table 1: Cultural characteristics and biochemical characteristics of bacteria isolated from the Skin of *Clarias gariepinus*

Samp le code	Cultural characteri stic	Gra m stai n	shap e	Catala se test	Coagul ase test	Motili ty	Sucro se	Gluco se	Galact ose	Fructo se	Manni tol	Malto se	Probable organism
CgS1	Yellow and white covered	+	Cocc i in chain	+	-	-	AG	AG	A-	A-	AG	A-	<i>Streptococ cus feacalis</i>
CgS2	Yellow tentate	-	Rod	+	+	-	AG	AG	AG	A-	AG	AG	<i>Xanthomo nas compestri s</i>
CgS3	Straight strand	-	com ma	+	+	+	AG	AG	AG	A-	AG	AG	<i>Virbio sp</i>
CgS4	White bulb- like	-	Rod	+	-	+	AG	AG	AG	A-	A-	AG	<i>Zoogloea ramigea</i>
CgS5	Tiny white	-	Cocc i	+	-	+	A-	AG	A-	--	A-	AG	<i>Micrococc us spp</i>
CgS6	Rhizoid flat	-	Rod	+	-	-	AG	AG	A-	--	AG	A-	<i>Citrobacte r freundii</i>
CgS7	Opaque	-	Rod	+	-	+	--	--	A-	AG	AG	A-	<i>Escherichi</i>

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CgS8	Circular opaque	-	Rod	+	+	+	AG	A-	A-	AG	AG	-G	<i>a coli</i>
CgS9	Rhizoid	-	Rod	+	+	+	--	A-	--	--	--	--	<i>Escherichia coli</i>
Cg10	Transparent milk	+	Rod	+	+	+	A-	-G	--	-G	A-	A-	<i>Proteus sp</i>
													<i>Lactobacillus planetarium</i>

KEY: A= Acid Production; AG= Acid and Gas Production; G= Gas Production; - - = No gas and Acid Production

Table 2: Cultural characteristics and biochemical characteristics bacteria isolated from the Intestine, gill and liver of *Clarias gariepinus*

Sample code	Cultural characteristic	Gram stain	Shape	Catalase test	Coagulase test	Motility	Sucrose	Glucose	Galactose	Fructose	Mannitol	Maltose	Probable organism
CgIN 1	Opaque	+	Rod	-	-	+	A-	A-	A-	AG	AG	AG	<i>Bacillus subtilis</i>
CgIN 2	Orpbia	-	Cocci	-	-	-	--	A-	A-	AG	AG	A-	<i>Aerococcus aerogenes</i>
CgIN 3	Small white	-	Spars e rod	+	-	+	-G	AG	AG	AG	AG	--	<i>Alcoligene s feacalis</i>
CgIN 4	Very tiny dot	-	Multiple rod	+	-	-	A-	AG	A-	A-	--	AG	<i>Aeromonas hydrophyla</i>
CgIN 5	Small white	+	Cocci	+	-	-	A-	--	--	AG	--	AG	<i>Streptococcus feacalis</i>
CgG 1	Rough, faint white	+	Cocci in chain	-	-	-	A-	--	A-	A-	AG	--	<i>Streptococcus feacalis</i>
CgG 2	Yellow, small, smooth	+	Spiral	+	-	+	AG	A-	A-	A-	AG	A-	<i>Spirillum graniferum</i>
CgG 3	Opaque	-	Rod	+	+	+	A-	A-	A-	AG	AG	AG	<i>Staphylococcus epidermidis</i>
CgG 4	Translucent white and milky	-	Cocci in cluster	+	+	-	A-	A-	A-	A-	AG	--	<i>Staphylococcus aureus</i>
CgG 5	White circular	+	Short rod	+	+	-	AG	A-	A-	A-	A-	AG	<i>Escherichia coli</i>
CgG 6	Circular faint white	-	Small rod	+	+	-	--	A-	--	--	AG	--	<i>Enreobacter aerogenes</i>
CgG 7	Large white	-	Spiral	-	+	-	--	--	A-	AG	AG	A-	<i>Spirillum graniferum</i>
CgG 8	Milky ,large, smooth	-	Short rod	+	-	-	AG	AG	AG	A-	AG	AG	<i>Fusobacterium sp</i>
CgL 1	White tentate	+	Rod in chain	-	-	-	AG	A-	AG	AG	AG	AG	<i>Bacillus cereus</i>
CgL 2	Brown	-	Rod	-	-	+	AG	A-	A-	A-	AG	A-	<i>Bacteriodes fragilis</i>

KEY: A= Acid Production; AG= Acid and Gas Production; G= Gas Production; - - = No gas and Acid Production

Table 3: Cultural characteristics and biochemical characteristics of bacteria isolated from the Skin, intestine, and liver of *Gymnarchus niloticus*

Sample code	Cultural characteristic	Gram stain	Shape	Catalase test	Coagulase test	Motility	Sucrose	Glucose	Galactose	Fructose	Mannitol	Maltose	Probable organism
GnS1	Strands, straight	+	spiral	-	-	-	AG	AG	AG	A-	AG	AG	<i>Campylobacter spurorum</i>
GnS2	Circular (round) yellow	-	Short rods in cluster	+	-	-	AG	AG	AG	AG	AG	AG	<i>Pseudomonas syringae</i>
GnS3	Dots (Numerous)	+	Cocci	+	-	-	--	A-	AG	--	AG	AG	<i>Staphylococcus sp</i>
GnS4	Circular yellow	+	Cocci in cluster	+	+	+	AG	AG	AG	AG	AG	--	<i>Staphylococcus aureus</i>
GnS5	Opaque	-	Rod	+	-	+	--	--	A-	AG	AG	A-	<i>Escherichia coli</i>
GnIN 1	Circular, white, smooth	+	Cocci in chain	+	-	+	AG	A-	A-	A-	AG	A-	<i>Streptococcus faecalis</i>
GnIN 2	Fairly milk	-	Cocci in cluster	+	-	-	AG	A-	A-	A-	A-	A-	<i>Staphylococcus aureus</i>
GnIN 3	Fair white	+	Cocci in chain	+	+	-	AG	A-	A-	A-	A-	A-	<i>Streptococcus faecalis</i>
GnG	Milky, largely circular	+	Short Rod	+	+	-	AG	--	A-	AG	AG	A-	<i>Escherichia coli</i>

KEY: A= Acid Production; AG= Acid and Gas Production; G= Gas Production; - - = No gas and Acid Production

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