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Full length Research paper

Evaluation of microorganisms on *clariasgariepinus* and *gymnarchusniloticus* from Delta region (Ese Odo), Southwest Nigeria

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A study of environmental conditions and effects on bacterial abundance, load, and prevalence of two economically important fish species in Nigeria (Clariasgariepinus and Gymnarchusniloticus) was conducted in the (aquatic) environment of the Ese Odo Delta. A total of 46 fish samples, including 23 Clariasgariepinus and 23 Gymnarchusniloticus, were collected from around the EseOdo Delta and analyzed for bacterial abundance, prevalence, and burden. 17 species of bacteria were recovered from African Clarias and 5 species of bacteria were recovered from Gymnarchus niloticus. The bacterial load in the body of Clarias garie was 67.28 ± 29.11 × 103 in the skin, 30.14 ± 29.16 × 103 in the intestine, 22.57 ± 9.57 × 103 in the gills, 11.71 ± 17.34 × 102 in the liver, and 60.29 ± 25 for Gymnarchusniloticus. , 69, 69 ± 25.69 × 103 skin, 11.14 ± 3.43 × 103 intestine, 5.17 ± 2, 05 × 103 gills and liver 3.14 ± 2.47 × 102. The prevalence of bacteria was highest in the skin. .. Bacterial species include Bacillus sp, Streptococcus sp, Spirillum sp, Pseudomonas sp. , Escherichia coli, Vibrosp, Proteus sp, Aerococcus sp, Lactobacillus sp, Micrococcus sp, Staphylococcus sp, Fusobacterium sp, Citrobacter sp, Bacteriodes sp, Zoogloe sp. , Alcoligene ssp and Xanthomonas sp. Staphylococcus sp is G. 33% in niloticus, C. Gariepinus accounted for 16%. There was also a significant difference in bacterial load and prevalence between C. gariepinus and G. niloticus fish collected from the Ese Odo Delta environment.

Keywords: Microorganisms, *Clariasgariepinus, Gymnarchusnilotics,* Esa-Odo, Environment, bacteria

INTRODUCTION

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

Gymnarchusniloticus (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.

MATERIALS AND METHODS

Live *Clariasgariepinus* and *Gymnarchusniloticus* 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 10^3dilution; spread on petri dishes containing nutrient agar prepared at 121^0C for 15 minutes; and incubated for 24 hours at 37^0C.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 4ºC.Identification of recovered bacterial was carried out using colonial, morphological and biochemical characteristics of colonies. Physical observation of surface colonies on nutrient agarmedium 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. Onepercent sugars such as glucose, sucrose, lactose, maltose, and others were used in abasal 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 andproduction of gas in Durham tube provided for the test. Recovered bacterial are counted to know most occurred and pre\vailed bacterial for health.

RESULTS AND DISCUSSION

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

Eighteen species of bacteria were recovered from the two fish species: *Clariasgariepinus* and *Gymnarchusniloticus*, 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, Vibrosp, Proteus sp, Aerococcussp ,Lactobacillussp, Micrococcus sp, Staphylococcus sp, Fusobacterium sp, Citrobacter sp, Bacteriodessp, zoogloeasp, Campylobacter sp, Alcoligenessp,* 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 (Table1and 2). *Baccilus* 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 Spirillium 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 *Bacteriodessp* in liver of *C.gariepinus. Staphylococcus sp*p revailed 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 food borne 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 (*Clariasgariepinus*). 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

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

Sample code	Cultural characteristic	Gram stain	shape	Catalase test	Coagulase test	Motility	Sucrose	Glucose	Galactose	Fructose	Mannitol	Maltose	Probable organism
CgS1	Yellow and white covered	+	Coccin chain	+	_	_	AG	AG	A-	A-	AG	A-	*Streptococcus feacalis*
CgS2	Yellow tentate	_	Rod	+	+	_	AG	AG	AG	A-	AG	AG	*Xanthomonas compestris*
CgS3	Straight strand	_	comma	+	+	+	AG	AG	AG	A-	AG	AG	*Virbiosp*
CgS4	White bulb- like	_	Rod	+	_	+	AG	AG	AG	A-	A-	AG	*Zoogloearamigea*
CgS5	Tiny white	_	Cocci	+	_	+	A-	AG	A-	--	A-	AG	*Micrococcus spp*
CgS6	Rhizoid flat	_	Rod	+	_	_	AG	AG	A-	--	AG	A-	*Citrobacter freundii*
CgS7	Opaque	_	Rod	+	_	+	--	--	A-	AG	AG	A-	*Escherichia coli*
CgS8	Circular opaque	_	Rod	+	+	+	AG	A-	A-	AG	AG	-G	*Escherichia coli*
CgS9	Rhizoid	_	Rod	+	+	+	--	A-	--	--	--	--	*Proteus sp*
Cg10	Transparent milk	+	Rod	+	+	+	A-	-G	--	-G	A-	A-	*Lactobacillus planetarium*

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 *Clariasgariepinus*

Sample code	Cultural characteristic	Gram stain	Shape	Catalase test	Coagulase test	Motility	Sucrose	Glucose	Galactose	Fructose	Mannitol	Maltose	Probable organism
CgIN1	Opaque	+	Rod	_	_	+	A-	A-	A-	AG	AG	AG	*Bacillus subtilis*
CgIN2	Orpbia	_	Cocci	_	_	_	--	A-	A-	AG	AG	A-	*Aerococcus aerogenes*
CgIN3	Small white	_	Sparse rod	+	_	+	-G	AG	AG	AG	AG	--	*Alcoligenesfeacalis*
CgIN4	Very tiny dot	_	Multiple rod	+	_	_	A-	AG	A-	A-	--	AG	*Aeromonas hydrophyla*
CgIN5	Small white	+	Cocci	+	_	_	A-	--	--	AG	--	AG	*Streptococcus feacalis*
CgG1	Rough, faint white	+	Coccin chain	_	_	_	A-	--	A-	A-	AG	--	*Streptococcus feacalis*
CgG2	Yellow, small, smooth	+	Spiral	+	_	+	AG	A-	A-	A-	AG	A-	*Spirillum graniferum*
CgG3	Opaque	_	Rod	+	+	+	A-	A-	A-	AG	AG	AG	*Staphylococcus epiderdimis*
CgG4	Translucent white and milky	_	Coccin cluster	+	+	_	A-	A-	A-	A-	AG	--	*Staphylococcus aureus*
CgG5	White circular	+	Short rod	+	+	_	AG	A-	A-	A-	A-	AG	*Escherichia coli*
CgG6	Circular faint white	_	Small rod	+	+	_	--	A-	--	--	AG	--	*Enreobacter aerogenes*
CgG7	Large white	_	Spiral	_	+	_	--	--	A-	AG	AG	A-	*Spirillum graniferum*
CgG8	Milky ,large, smooth	_	Short rod	+	_	_	AG	AG	AG	A-	AG	AG	*Fusobacterium sp*
CgL1	White tentate	+	Rod in chain	_	_	_	AG	A-	AG	AG	AG	AG	*Bacillus cereus*
CgL2	Brown	_	Rod	_	_	+	AG	A-	A-	A-	AG	A-	*Bacteriodes fragilis*

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 *Gymnarchusniloticus*

Sample code	Cultural characteristic	Gram stain	shape	Catalase test	Coagulase test	Motility	Sucrose	Glucose	Galactose	Fructose	Mannitol	Maltose	Probable organism
GnS 1	Strands, straight	+	spiral	_	_	_	AG	AG	AG	A-	AG	AG	*Campylobacter spurorum*
GnS 2	Circular (round) yellow	_	Short rods in cluster	+	_	_	AG	AG	AG	AG	AG	AG	*Pseudomonas syringae*
GnS 3	Dots (Numerous)	+	Cocci	+	_	_	--	A-	AG	--	AG	AG	*Staphylococcus sp*
GnS 4	Circular yellow	+	Cocci in cluster	+	+	+	AG	AG	AG	AG	AG	--	*Staphylococcus aureus*
GnS 5	Opaque	_	Rod	+	_	+	--	--	A-	AG	AG	A-	*Escherichia coli*
GnIN 1	Circular, white, smooth	+	Cocci in chain	+	_	+	AG	A-	A-	A-	AG	A-	*Steptococcusfeacalis*
GnIN 2	F airly milk	_	Cocci in cluster	+	_	_	AG	A-	A-	A-	A-	A-	*Staphylococcus aureus*
GnIN 3	Fair white	+	Cocci in chain	+	+	_	AG	A-	A-	A-	A-	A-	*Streptococcus feacalis*
GnG	Milky, largely circular	+	Short Rod	+	+	_	AG	--	A-	AG	AG	A-	*Escherichia coli*

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

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.

CONCLUSION

The investigation on occurrence of bacterial flora on *Clariasgariepinus* and *Gymnarchusniloticus* 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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