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ORIGINAL ARTICLES

Isolation and Identification of Aerobic Bacteria Flora of the Skin and Stomach of Wild and Cultured *Clarias Gariepinus* and *Oreochromis Niloticus* from Ibadan, Southwest Nigeria

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ABSTRACT

Bacteria flora of wild and cultured *Clarias gariepinus* (African catfish) and *Orepchromis niloticus* (Nile Tilapia) randomly collected from different aquatic environments in Ibadan southwest Nigeria were examined and compared. 210 tissue samples harvested from skin ($1cm^2$) and stomach (1g) were aseptically analysed. Samples were cultured for bacteria using standard methods. The bacteria species isolated are those in genera *Bacillus, Proteus, Pseudomonas, Klebsiella, Streptococcus, Salmonella, Staphylococcus, Micrococcus, Serratia and Escherichia*. The highest number of different bacteria count was recorded in tilapia species captured from natural river with mean bacteria count (6.09±0.65, $t<0.05$). Escherichia coli was most frequently encountered (13.06%). The bacteria isolated were of public health significance and the implications of these observations are discussed.

Key words: Public Health**,** aquaculture parasites.

Introduction

The aquaculture industry in Nigeria (Federal department of Fisheries, 2007) mainly involves African catfish (*Clarias gariepinus*) and Tilapia species (*Oreodotuus niloticus*) production. Intensive production of fish increases the likelihood of and severity of parasite and disease outbreaks which constitutes a major constraint to aquaculture production (Yunvia *et al*, 2001) and linkages have been made between fish production and public health in terms of communicable diseases, non-communicable disease, malnutrition and injury (Binkey and Lock, 1998). Though poisons or death from fish consumption is not widespread in Nigeria and information on pathogen, natural and spoilage bacteria flora of fish is severely limited (Korie-Siakpere and Evbakhare 1992; Efiuvwere and Ajiboye 1996; Molokwu and Okpokwasil, 2002) compared with other region of the world (Babu 2000, Spangard *et al*, 2000). The public health importance of bacteria flora of Nigeria fish species has not been adequately defined. This could be due to mode of food preparation which includes cooking for considerable length of time (Sowumi *et al*, 2008). The presence of various organisms which are particularly pathogenic to human in fish is only suggestive, its significance in initiation of human disease is unknown (Ligia *et al*., 2003). However, the presence of potential human pathogens suggests the fish improperly handled, undercooked or consumed raw may cause disease to susceptible individuals. This study tend to provide information on bacteria flora from the skin and stomach of *Clarias gariepinus* (African catfish) and *Oreochomis niloticus* (Nile Tilapia) both of which support huge artisanal and culture fisheries in Nigeria, highlighting their public health importance.

Materials and methods

Study Location:

Three study sites in Ibadan, Southwest Nigeria were used for this study (Table 1). A commercial fish pond, (A), A fishery institute fish ponds (B), and a River (C). The area and depth of the ponds were determined.

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Table 1: The Descriptive analysis of the study sites.

Sites	*Latitude	*Longitude	Area (m^2)	Water depth (m)
A	7.3878 N	3.8964 E	120,270	1.1
B	7.3878 N	3.8964 E	20,045	1.0
C	7.3878 N	3.8964 E	NA	NA

A, commercial fish pond; B, pond of fishery institute; C, Eleyele river, NA- Not available *Source: http// www.Wikipedia.com/june 2010.

Collection and Processing of Fish Samples:

Live African catfish (*Clarias gariepinus)* and tilapia species (*Oreochromis niloticus)* were randomly collected from the study sites. Wild African catfish and wild tilapia fish were collected from Eleyele River, while cultured African catfish and cultured tilapia fish were collected from ponds of the commercial farm and the fisheries institute respectively. A total of 210 tissue samples (skin and stomach) harvested from 48 fishes (24 *Clarias gariepinus*- 12 wild and 12 cultured), with average weight (grams) of 814 ± 82.95 and 1146.67 ± 36.98 for wild and cultured respectively (24 *Oreochromis niloticus* – 12 wild, and 12 cultured) with average weight (grams) of 99.83 ± 21.76 and 65.63 ± 9.6 for wild and cultured respectively were analyzed in this study. Eighteen (18) samples of feral (natural) and cultured pond water were randomly collected from different locations and analyzed. Fish were caught by a local fishing gear and by cast net. Sampling was drawn between 8.00 and 10.00 am in each occasion at periodic intervals of seven days for three consecutive times. Fish samples were transported directly to the Food and Meat Hygiene Laboratory of Department of Veterinary Public Health and Preventive Medicine, University of Ibadan within 2hrs of sampling.

Sample Preparation:

Bacterial isolates from each specimen were obtained from skin and stomach tissue samples by macerating aseptically skin ($1cm^2$) and stomach (1g portion) separate and shaking in 10ml distilled water. The stock solution was serially diluted ten folds. 0.1ml of (10^{-10}) dilution was spread on to nutrient agar and MacConkey Agar (MCA) in duplicate and incubated for 18-24 hrs at 37^0C. The bacteriological media namely nutrient agar (NA) and MacConkey Agar (MCA) (Micrometer, Theme, India) were prepared according to manufacturer's instructions. The media was sterilized at 121^0C for 15 minutes in an autoclave (Fishers scientific, USA) and was poured into sterile disposable petri dishes (Fishers scientific).

The colony forming counts per $1cm^2$ for skin and per 1gm of stomach was determined using standard methods (Horsely, 1977, APHA, 1995).

The results obtained were converted to logarithms in base ten. Each distinct colony was further subcultured on freshly prepared NA and MCA for evaluation of purity and colonial morphology. The isolates were then identified using gram staining, physiological, biochemical reaction and fermentation of sugars according to standard taxonomic schemes (Burchanan and Gibbons, 1974).

Results and discussion

The results of the means of morphometric parameters of the fish samples are as shown in Table 2. While the mean weight of 814g was obtained for wild *C. gariepinus*, the mean weight of 1146.67g was obtained for the cultured *C. gariepinus*. However, in the case of *O. niloticus*, the mean weights of 99.83 and 65.63g were obtained for the wild and cultured fish respectively. The means of weight and standard length of cultured *C. gariepinus* were significantly higher than the wild *C. gariepinus.* However, there was no significant difference in the weight and the standard length of the wild and cultured *O. niloticus.*

The range of bacterial isolates obtained from the stomach and fish samples is shown in table 3 with 9 different bacteria strains isolated from skin samples while 5 different types were isolated from stomach samples. Species of bacteria isolated are in genera *Bacillus, Proteus, Pseudomonas, Klebsiella, Streptococcus, Salmonella, Staphylococcus, Micrococcus, Serratia and Escherichia*.

The predominant microorganisms isolated from parts of the fish are shown in percentage in Table 4. Escherichia coli (13.06). Bacllus sp (12.24%). Proteins sp (10.61%) and Pseudomus (10:20%) are most frequent isolated bacteria strains. In both species, wild Tilapia recorded highest bacteria count.

Table 2: The morphometric parameters of *C. gariepinus* and *O. niloticus* (wild and cultured types).

*Morphometric Parameter	*Clarias gariepinus*			*Oreochromis niloticus*		
	Wild	Cultured	t*	Wild	Cultured	t*
Weight (g)	814± 82.95	1146.67± 36.98	-3.66**	99.83± 21.76	65.63± 9.26	1.45
Standard length (cm)	40.82± 4.20	55.42±1.31	-3.25**	16.01± 1.45	19.55± 2.38	-1.27
STD (weight)	287.00	127.96		75.3	32.04	
STD (length)	14.52	4.54		5.02	8.24	

STD, Standard deviation; *t, t-test value; **, Significant at P<0.05; *, each value is a mean of 12 readings

Table 3: Bacterial isolates obtained from different parts of fish.

Samples	Bacterial isolates
Skin	Bacillus sp, Klebsiella sp, Micrococcus sp, Staphylococcus aureus, Staphylococcus sp, Serratia sp, Proteus sp, Pseudomonas sp, and Streptococcus sp.
Stomach	Klebsiella sp, Serratia sp, Escherichia coli, Pseudomonas sp, and Salmonella sp.

Table 4: Distribution of bacteria isolates on *Clarias gariepinus* (wild and cultured) and *Oreochromis niloticus* (wild and cultured).

Isolates	Wild	Cultured	Wild	Cultured	**Total (%)
Bacillus sp	7(11.86)	6(9.52)	9(13.43)	8(14.29)	30(12.24)
Klebsiella sp	5(8.47)	7(11.11)	2(2.99)	6(10.71)	20(8.16)
Proteus sp	6(10.17)	8(12.70)	8(11.94)	4(7.14)	26(10.61)
Salmonella sp	5(8.47)	7(11.11)	3(4.48)	6(10.71)	21(8.57)
Staphylococcus aureus	3(5.08)	5(7.94)	7(10.45)	5(8.93)	20(8.16)
Staphylococcus sp	2(3.38)	4(6.35)	6(8.96)	3(5.36)	15(6.12)
Pseudomonas sp	6(10.17)	8(12.70)	5(7.46)	6(10.71)	25(10.20)
Serratia sp	4(6.78)	3(4.76)	7(10.45)	2(3.57)	16(6.53)
Micrococcus sp	3(5.08)	3(4.76)	5(7.46)	5(8.93)	16(6.53)
Streptococcus sp	7(11.86)	6(9.52)	7(10.45)	4(7.14)	24(9.80)
Escherichia coli	11(18.64)	6(9.52)	8(11.94)	7(12.5)	32(13.06)
Total	59 (100)	63(100)	67(100)	56(100)	245(100)
Mean ± SEM	5.36±0.75	5.73±0.54	6.09±0.65	5.09±0.53	
t static	-0.398	1.190			

**% = Cummulative percentage
*Multiple isolates occurred in some samples
%(in brackets)

Discussion:

Most of the organisms observed in this study are pathogens capable of causing a variety of disease in man. Staphylococcus, gram positive facultative anaerobic bacteria, is widespread among mammalians where they belong to the health microflora of skin and mucosa. The coagulase positive species Staphylococcus aureus are the species with the broadest pathogenic potential causes infection of the skin, deeper tissues and organs, pneumonia, enteritis and pseudomembranous enterocolitis and food poisoning. In contrast to S. aureus, members of the heterogenous group of coagulase-negative staphylococci (CNS) are regarded as less pathogenic bacteria. CNS indeed represents a substantial part of the saprophytic microflora in humans and they rarely cause disease in immunocompetent outpatients. In recent decades, however, coagulase negative staphylococci have emerged as nosocomial pathogens in immunocompromised individuals. Specifically, *Staphylococcus epidermidis* is a common cause of line-associated septicemia and other polymer-related infections. Noscomial isolates of both S aureus and CNS are characterized by increasing resistance towards antibiotics which is a great challenge for the management of hospital-acquired infections (Ziebhur, 2001).

Escherichia 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. E. coli causes diarrhea, urinary tract and kidney, infections and peritonitis septiceamia. Where animal manure, particulars bovine manure, is used as pond fertilizers, there is a risk that pathogenic strains of E. coli may be present in pond water. For instance there is a good evidence for the occurrence of waterborne infection caused by E. coli 0157: H7. As the occurrence of this strain in cattle is well established and its infections dose is low, it poses a potential risk to public health where bovine manure is used as pond fertilizer (WHO, 1997).

Salmonella spp. are among the most important causes of human gastrointestinal disease worldwide and many seafood-importing countries will not accept products containing these pathogens. Studies have indicated that there is a higher prevalence of salmonella in tropical than in temperate waters and although seasonal variations do occur. Salmonella spp may be naturally present in same tropical aquatic environments. It is well established that aquatic birds spread these organisms and other pathogen in the environment (Felon, 1983; Beveridge, 1989). Salmonella spp tend to be associated with the intestinal tracts of warm-blooded animals, they have also been detected in the gut of tilapia and Carp grown in waste-fed and non-waste-led aquaculture ponds (Buras, 1993, Iyer and Shrinvastava, 1989).

Serratia spp causes bacteriuria while Pseudomonas had been isolated in wounds, burns, eyes and ears infection (Meyer 1974). Many pathogens species encountered in this study are no doubt potentially pathogen to human. Bacllus sp. E. coli, Salmonella sp. Streptococcus sp and S. aurens were also implicated in fish-borne (Babu 2000), strimp-borne (Raghavan, 2003) diseases of humans. The public health importance of bacterial flora of Nigeria fish species have not been adequately defined due mainly to mode of food preparation in the tropic which involved cooking for considerable length of time. The heat would have eliminated most, if not all the bacterial flora (Sowumi *et al*., 2008).

The isolation of these pathogens from fish samples is worrisome because of their potential in causing ill-health in human. It is noteworthy to assume that these pathogens might be introduced into the production process by human healthy carriers through handling. The continuous contamination of the process may be enhanced through the processing equipment (Hatcher *et al*., 1992). Stress, and consequently immune suppression is probably the commonest underlying cause of disease in fish. Usual pathogens such as Aeromones, Pseudomonas and Flavobacteria are environmental contaminants, and usually secondary invaders of otherwise stressed fish. Proteus sp and streptococcus sp have been linked with certain disease conditions in tilapia. Streptococcus pyogenes was associated with infected liver and intestines (Balarin, 1979) and Proteus retigari implicated in huge loss of stock (Sagua, 1986). Both infections were associated with presence of organic manure from either human or non-human sources. These bacteria species may therefore be opportunistic. Association of bacteria with specific fish disease has not been successful in C. *gariepinus* and Tilapia. spp. as C. gariepinus is regarded as a rather resistant fish (Huisman and Richter, 1987). Boon and Huisman (1986) observed that despite the tremendous expansion been witnessed in commercial fish farming in Nigeria, a number of fish diseases still remain unknown.

In different studies, Ekundayo 1977 on Lagos lagoon, Ajiwe *et al* (2000) on Ele river and Ibe and Ozor (2000) on Otamiri 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.

In view of the fact that bacteria isolated in the course of our study are potentially pathogenic to human and may be associated with specific fish disease. It is therefore recommended that the sanitary conditions under which fishes are reared in ponds should be improved, following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things. The farmers should embrace standard operating practices as applicable to fish farming. The workforce should be educated on the maintenance of good hygienic practices and should be provided with necessary working and safety equipment. The microbial quality of fish can also be improved through regular disinfection of catching gears or working equipment and brief immersion of caught fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public. The public should be enlightened on the inherent danger that may accompany handling of fresh fish or consumption of improperly cooked fish. Therefore, fish must be properly cooked before it is consumed to avoid contact with the microbes that may be associated with it.

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