Bacteriological analysis of recirculatory Aquaculture systems in some fish farms in Lagos, Nigeria

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Abstract: Bacteriological analysis of recirculatory aquaculture systems in eight farms randomly selected in the Lagos metropolis was carried out. Two fish farms each were examined from Iyana-Iba, Victoria Island, Maryland and Ibeju Lekki. A set of questionnaires was also administered on the staff of the fish farms. Ninety six (96) catfish, *Clarias gariepinus* were obtained from the fish farms studied with twelve fish samples from each of the fish farms. Each fish was dissected aseptically to bring out the liver and kidney. A swab each of the liver, the kidney, the gill and the skin were studied bacteriologically using standard methods. Water samples and feeds were also examined from all fish farms for *E.coli* and other enteric bacterial pathogens. Thirteen bacterial species were recovered from the fish farms which included *E.coli*, *Shugella* sp, *Edwardsiella* sp, *Enterobacter* sp., *Pseudomonas sp,. Aeromonnas* sp., *Pleisomonas* sp., Vibrio sp., *Hafnia* sp., *Acinetobacter* sp, *Serratia* sp, *Salmonella* sp and *Yersinia* sp. No bacteria was recovered from the fish feed and the stocking density practiced in all fish farms conformed with standard practices. Educational status appeared not to be a predisposing factor to fish infection.
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1. Introduction

A recirculatory aquaculture system (ARS) is a production system that recirculates the water rather than passing it through only once. Less water is needed for this production system than for ponds or concrete tanks. This system enjoys complete environmental control and all year round availability of controlled harvest of fish, but it is quite new in Nigeria and accounts for less than 10% of culture practices (Omitogun, 2007).

Aquaculture, the rearing of aquatic organisms has high prospects in Nigeria with a population of over 140 million people which has a high fish demand (Akindele, 1989). Aquaculture therefore remains the only viable alternative for increasing the fish protein needs of our people and Nigeria is blessed with suitable land, freshwater, brackish and marine fish species which can be cultured.

In recent aqua-cultural activities, fish diseases have become a recurrent situation to fish farmers across various fish farms worldwide. Diseases can be introduced into RAS from water, the fish, the feeds and the systems equipment (Ronald, 1998). The impact of infectious disease remains generally one of the limiting factors to successful economic production of fish worldwide (Masser et al, 1999). There is an increasing awareness that stressful environmental husbandry practices and/or infection of fishes by other microbes can suppress the immune system of healthy fish, which increases the risk of disease caused by infectious microbes such as bacteria, fungi, viruses and parasites (Omitogun et al, 2000). The interaction between fish and pathogenic organisms that are present in aquatic environment are a potentially serious source of mortality resulting in economic loss.

This study was carried out to assess fish disease in RAS in some randomly selected areas in the Lagos metropolis, to identify the predisposing factors to infection of fish in RAS and to assess the quality of personnel employed in the RAS. Furthermore, bacterial pathogens from RAS was isolated, identified and characterized. The effect of such pathogens on the food handlers and their public health significance was discussed.

2. Materials and Methods

2.1 Study Area

Ninety six (96) cat fish (*Clarias gariespinus*) were obtained from a total of eight fish farms represented by two farms each from Victoria Island , Ibeju Lekki Maryland and Iyana Iba, within the Lagos metropolis of Nigeria.

2.2 Sample Collection

The Fish samples were captured alive into eight 25-litres kegs, with half of the top cut for aeration. Water samples and feed were collected aseptically from each farm and were taken to the microbiology

laboratory at the Lagos State University, Ojo, Nigeria for prompt analysis.

2.3 Bacteriological Analysis

Each fish sample was dissected aseptically to remove the liver and kidney. Swabs from the gill and the skin as well as the kidney and liver, ground in mortar aseptically, were inoculated on Salmonella-Shigella agar (SS) MacConkey agar and Eosin Methylene Blue agar (EMB). The plates were incubated at 37C for 18 – 24 hours. All bacterial colonies were subjected to Grams staining and other biochemical tests after incubation, and identified according to Barrow and Feltham, 1995 (Barrow & Feltham, 1995).

2.4 Questionnaire

A set of Questionnaire was designed to assess the status of RAS vis-a-vis disease, personnel, water quality, feed quality and type of treatment, where applicable.

2.5 Statistical Analysis

One way analysis of variance (ANOVA) was used to test the significance among the species composition in each RAS sites.

3. Results

Bacteriological analysis fish of skin, gills and kidney of 96 catfish (*Clarias gariepius*), as well as the feed and water samples from eight RAS farms showed the presence of several bacterial pathogens such as *Escherichia coli*, *Shigella* sp., *Salmonella* sp., *Enterobacter sp., Pseudomonas sp*., *Aeromonas* sp., *Pleisomonas* sp., *Edwardsiella* sp., *Hafnia* sp., *Acinetobacter* sp., *Serratia* sp. and *Yersinia* sp. The organisms were identified by cultural, morphological and biochemical characteristics according to Barrow and Feltham, 1995 (Table 1).

Table 1: Bacteria recovered from different sample sites (A-H).

Bacteria	A	B	C	D	E	F	G	H
Salmonella sp.	+	+	+	+	+	+	+	+
Edwardsiella sp	+	+	+	+	+	+	-	-
***Enterobacter* sp**	+	+	+	+	-	-	-	-
***Hafnia* sp.**	+	+	-	-	+	+	+	+
***Serratia* sp.**	+	+	+	+	-	-	-	-
***Yersinia* sp.**	+	+	+	+	-	-	-	-
***Acinetobacter* sp.**	+	+	+	+	-	-	-	-
***Pseudomonas* sp.**	+	+	+	+	+	+	-	-
***Aeromonas* sp.**	+	+	+	+	+	+	+	+
***Pleisomonas* sp.**	+	+	+	+	-	-	-	-

Key
+ =Present
- = Not present

More bacterial isolates were recovered from the kidney and gills as against the liver and the skin (Table 2).

Table 2: Distribution of bacteria in the kidney, liver, gill and skin.

Fish farm	Kidney	Liver	Skin	Gill	Total
A	**6**	**2**	**1**	**3**	**12**
B	**4**	**2**	**2**	**2**	**10**
C	**8**	**4**	**2**	**6**	**20**
D	**8**	**6**	**2**	**8**	**24**
E	**12**	**4**	**5**	**5**	**26**
F	**8**	**6**	**3**	**7**	**24**
G	**9**	**4**	**9**	**5**	**27**
H	**7**	**6**	**3**	**7**	**23**
Total	**62**	**34**	**27**	**43**	**166**

A study of the educational qualification of the farm managers showed that only two of them had Doctor of philosophy (PhD); two had either bachelor of science (B. Sc.) or Higher National Diploma, and 2 of them had ordinary national diploma (OND). The remainders did not respond, and this may likely be due to illiteracy. Although, the farm managers claim to check the water parameters, a high level of ammonia and nitrite were observed. A study of the stocking

density at each of RAS farm showed that stocking ranges between 200/m^3 to 400/ m^3.

Table 3: Stocking density of the RAS farms.

Fish farms	A	B	C	D	E	F	G	H
200/M^3	-	-	-	-	+	+	-	-
300/m^3	+	+	-	-	-	-	-	-
350/m^3	-	-	-	-	-	-	+	+
400/m^3	-	-	+	+	-	-	-	-

A generally low compliance with stocking density guidelines was observed in most fish farms, which is below standard.

4. Discussion

Ten bacterial species were recovered in this study which included *Salmonella sp*., *Edwardsiella* sp., *Enterobacter* sp., *Hafnia* sp., *Serratia* sp., *Yersinia* sp ., *Acinetobacter* sp., *Pseudomonas* sp., *Aeromonas* sp. and *Pleisomonas* sp. Several studies (Davis et al, 1967, Ogbondeminu, 1993; Ogbondeminu et al, 1999), reported high occurrence of similar bacteria. *Salmonella* sp. and *Aeromonas* sp. were the predominant organisms in this study which disagrees with Ogbondeminu, 1993, who reported *Pseudomonas* sp and *Aeromonas* sp. as the most dominant bacterial species associated with fish culture. The disparity may be due to differences in sampling techniques, source of samples and analytical methods in these studies.

The presence of bacterial pathogens in most of the RAS sites points to the fact that the rate of bacterial infection is very high, the consequence of which will be high morbidity and mortality rates in such fish farms. Fish farmers using RAS may therefore experience decrease in projected profit. This is of very serious economic consequences which may likely undermine the significance of RAS. Such economic losses due to infection by *E. tarda, E. ictaluri, A. hydrophila* and *A. salmonicida* have been reported (Masser et al, 1999, Ronald, 1998). No Gram-positive bacterium was isolated from this study as against the report of (Ogbadu & Okolo, 1993) who recovered *S. aureus* and *Streptococcus* spp. from fish (Ogbondeminu et al, 1999). Their findings however agree with this study in that majority of their bacterial isolates were Gram-negative bacteria (Ogbadu & Okolo, 1993, Ogbondeminu et al, 1999, Wiklond & Bylund, 1990).

Most of the bacteria isolated from this study cause different diseases of fish (Inglis et al, 1994). The high prevalence of these pathogens and opportunistic bacterial pathogens may account for high prevalence of diarrhea and other food borne infections in developing countries, including Nigeria. Human infections that can be caused by bacteria in fish and man are mostly food poisoning and gastroenteritis in which organisms such as *Salmonella* spp., *Vibrio* spp., *Clostridium* spp. and *Campylobacter jejuni* have been implicated (Inglis et al, 1994; Ugwuzor, et al, 1990). .*Pseudomonas* spp. cause superficial conditions such as wound infections but organisms often implicated in diarrhea of fish include *Edwardsiella* spp., *Staphylococcus* spp. *Escherichia* spp and *Aeromonas* spp. (Inglis et al, 1994).

The most common diseases in recirculatory systems caused by bacteria and protozoans which have been particularly problematic in RAS include protozoal diseases like ichthyphthirius and trichodina, and bacterial diseases, caused by *Aeromonas*, *Streptococcus* and *Mycobacterium spps.* (MASSER et al, 1999). The high prevalence of bacteria in this study suggests cautious handling and regular sampling of the fishes in RAS in order to monitor and reduce the prevalence of such enteric bacteria, which are a likely source of public health hazard. This agrees with earlier reports by Inglis *et al*. (1994) and Masser *et al*. (1999).

All the bacteria recovered in this study are members of the family Entorobacteriaceae, hence may be traceable to the humans involved in handling the feeds. This became evident when all the feeds analyzed were devoid of bacterial contamination which suggests that the source of contamination is extraneous, and not likely from the feeds. This agrees with a report (Masser et al., 1999) that diseases can be introduced into the system from the water, the fish and the system's equipment. This could be due to aseptic production and packaging processes and the result of good feed storage conditions in the RAS sites.

Samples from sites A, B, C and D had the highest prevalence as against sites E and F with the least bacterial prevalence. This may perhaps be due to a better compliance with guidelines for RAS operators in sites in E and F (Inglis et al., 1994; Masser et al., 1999). This can as well be attributed to treatment problems, especially biochemical treatment problems which severely disrupt bio filters, which are usually inhibited to some degree by formalin, copper sulphate, potassium permanganate and certain antibiotics (Oladosu, 1998).

The high prevalence of bacterial pathogens in the kidneys, livers and gills in this study is quite significant. This is a clear evidence of RAS

contamination. These pathogens can easily be carried to other organs and tissues as they are associated with various septicaemic conditions which results in death of fishes (Ogbadu & Okolo, 1993). If such fish food is inadequately cooked, their consumption may be a source of food borne infections in humans (Ikpi et al., 2005). Stocking capacity in all farms appears not to be of any negative consequence as only 2 of the sites studied had bacteria and this appears to fall within the standard range. This finding agrees with report by Masser *et al (*1999*).*

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