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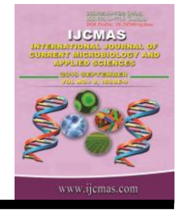

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Impact of Integrated Fish Farming on Antimicrobial Resistant Bacteria in Pond Environments in Osun State, Nigeria

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A B S T R A C T

The use of antimicrobial agents in integrated fish farms poses public health problem in a developing country like Nigeria. Materials used in this study were integrated fish farm, imported fish feed, pond water and fish guts respectively. Different bacteria were isolated from each material above and each isolate was characterized. Antibiotic susceptibility test was carried out on each isolate using commercially available antibiotic disks respectively. A total of 48 bacteria were isolated from the four samples. Pond water sample and pond sediment had a total of 14 (29.2%) bacteria isolates from Ringroad, Sawmill and Mobi respectively. Fish guts had a total of 11 (22.9%) of bacteria isolates from the three samples used. Lastly, 9 (18.7%) bacteria were isolated from the fish feed used in this study. Eleven species were isolated from the four specimen types. *Escherichia coli* occurs 7 (14.5%) in the four specimens while *Aeromonas species*, *Salmonella species* and *Morganella morgani* occur 5 (10.41%) in all the four sample types. Also, *Proteus species*, *Enterobacter sp*, *Klebsiella sp* and *Serratia* occur in all the four samples 4 (8.33%) while *Provindencia sp*. and *Shigella* sp occur 3 (6.25%) and 2 (4.16%) respectively. The result of antimicrobial susceptibility test by disk diffusion showed that Septrin (93%) and Chlorampericol (93%) had the highest resistance to all the bacteria isolates. This is followed by antibiotic resistance of Augumentin (67%) and Amoxacilllin (53%) respectively. However, this shows that there is need to evaluate the prevalence of antimicrobial resistant bacteria in integrated fish ponds in order to prevent and control the development of antibiotic resistant bacteria in pond environment.

Introduction

Integrated fish farming is a system of farming that combines livestock production with fish farming. The system utilizes animal excreta, urine and feed leftovers as pond substrates to enhance the growth of plankton and other microorganisms eaten by fish. In integrated fish farms, animal dung is shed into the fish pond as manure supporting the growth of plankton and other micro organisms eaten by fish thereby producing high yields of fish with low input as the fish receive limited supplementary feed. The livestock on the other hand are fed with feed containing antimicrobial agents which serve as growth promoters. It has the capacity of meeting up with fish demand as it supplies enough manure to produce large quantity of fish (Ayinla 2003). Integrated fish farming provides job opportunities to both the women and the teeming population of the youths in a country. In addition to the above, the system provides food security and raises nutritional status by providing important complementary ingredients for better nutrition.

The combination of fish and poultry or fish and piggery excreta have been found to fertilize the pond and are avenue for fish to derive their energy. The use of poultry in integrated fish farming has been found to be beneficial to the fishes because of the algal bloom produced which serves as food and hiding place for fishes (FAO, 2003). Pig and fish combination on the other hand increases both economic and ecological efficacy as aquatic plants, wastes, residues and left over from kitchen are used as Pig food while the excreta of the Pigs which is very rich in nutrients is used as organic manure and fish feed in ponds thereby greatly reducing the cost of fish production (AIFP. 2005).

The feed of the livestock mainly chickens and pigs contain antibiotics which are used for therapeutic or prophylactic purposes in integrated fish farms, These antimicrobial agents serve as growth promoters to fishes and prevent proliferation of bacteria in fish pond however bacteria that survive the antimicrobial agents become drug resistant serving as reservoirs of resistant genes from which genes can be disseminated to human pathogens (Ole *et al.*, 2009). These antibiotic resistant bacteria (ARB) may be harbored on the skin, gut and slime of living fish which if consumed may cause infections in humans and result in treatment failures.

Information on the development of antimicrobial resistant strains of bacteria in fish ponds in Osun state, Nigeria is scarce hence the need for this study which is designed to investigate the impact of integrated fish farming on antimicrobial resistant bacteria in a pond environment.

Materials and Methods

All materials including media, glass wares and bench surfaces were adequately sterilized.

Study area

Integrated fish farms located at Mopo Ede, Mobi Ede and Ring road Osogbo, Osun state, Nigeria were accessed in this study after consent was obtained from the owners. Information on the type of feed given to the fish and livestock was obtained verbally from the managers of the ponds.

Collection of samples

A total number of four specimens from each farm site were collected. The samples were; Pond sediments, Pond water, Fish feeds ad Fish gut. The samples were transported to the laboratory for immediate analysis.

Isolation of bacteria

One gram of Pond sediments, Pond water, Fish feeds and Fish guts was accurately weighed out, ground and serially diluted. One milliliter of the aliquots from the dilution 10^{-5} of each sample was transferred into appropriately labeled petri dishes into which sterile MacConkey agar was poured and allowed to solidify. This was done in triplicates **(**Harley and Precott, 1996**). **The plates were incubated at 37ºC for 24 hours after which developed colonies were counted to obtain total viable count followed by sub culturing. Pure cultures were stored at 4ºC in slants of MacConkey agar.

Characterization and identification of bacteria isolates

The isolates were characterized and identified based on their cultural characteristics, grams stain and biochemical reactions.

Antibiotic susceptibility test

The bacteria isolates were examined for susceptibility to commonly used antimicrobial agents using commercially available discs and following the manufacturers' instruction. The zones of inhibition were read after incubation and recorded as resistant or sensitive.

Results and Discussion

The average plate counts of samples from different location were significantly different as P < 0.05 for each of the locations. The bacterial count in Sawmill pond ranged from 5.0 x 10^6 Cfu/ml to 1.40 x 10^7 Cful/ml. Also, in Mobi pond the bacteria counts ranged from 9.5 x 10^6 Cfu/ml to 2.92 x 10^7 Cfu/ml while the bacteria counts in Ring road had 9.1 x 10^6 Cfu/ml to 2.27 x 10^7 Cfu/ml respectively. Pond sediments and water samples from Mobi pond had the highest bacterial counts and this was followed by those from fish pond water of Ring road and Sawmill respectively. The highest bacterial count was also recorded in Fish gut sample obtained from Sawmill pond water and this was followed by the higher bacterial count in Mobi and Ring road pond water respectively. Similarly, the highest bacterial count was recorded in Ring road pond followed by the least bacterial count in Mobi and Sawmill respectively (table 1).

A total of 48 bacteria isolates were gotten from four specimen types; Pond water, Pond sediment, Fish feed and Fish gut. Bacterial isolates identified were: *Escherichia coli* 7(14.58%), *Aeromonas species* 5(10.41%), *Salmonella species* 5(10.41%), *Proteus species* 4(8.33%), *Citrobacter freundii* 3(6.25%), *Morganella morganii 5*(10.41%), *Enterobacter species* 4(8.33%), *Serratia species* 4(8.33), *Klebsiella species* 4(8.33%), *Provindencia species* 3(6.25%) and *Shigella species* 2(4.16) (Table 3).

Escherichia coli, Aeromonas species and *Serratia species,* were the bacteria that occurred in all the four samples (Table 3). The bacteria isolates that occurred both in pond water and pond sediment were: *Citrobacter freundii* and *Proteus species* while only *Shigella species* were isolated from both fish gut and fish feed (Table 3).

Pond water had a total of 14 (29.2%) bacteria isolates of which 4 (8.33%) were from Ring road, 8(16.66%) bacteria isolates were from Mobi pond while 2(4.17%) bacteria isolates were from Sawmill pond at the lowest proportion. Water sediment had a total of 14(29.2%) bacteria isolates of which 2(4.16%), 9(18.7%) and 3(6.25%) were from Ring road, Mobi and Sawmill ponds respectively. Fish gut had a total of 11(22.9%) bacteria isolates of which 5(10.41%) and 3(6.25%) bacteria isolates were from Mobi, Ring road and Sawmill ponds respectively.

A total of 9(18.7%) bacteria isolates were reported for Fish feed out of which 2(4.16%) bacteria isolates were from Ring road, 4(8.33%) from Mobi and 3(6.25%) from Sawmill pond (Table 2).

The highest percentage of bacteria was observed to occur in water sediment sample. This could be as a result of accumulation of surplus feed overtime. Pond water sample was observed to be next in bacteria percentage. This could be because feed was applied to water over a period of time. Gut and feed samples had the lowest percentage of bacteria isolates. The reason could be because there was no accumulated effect in feeds and the feed which the fish ate had been mixed with water before entering the gut of the fish (Figure 1).

The result of the Antimicrobial Resistivity Test revealed that most of the isolated organisms were resistant to multiple antibiotics. The highest antimicrobial resistance of 93.33% was exhibited against Septrin and Chloramphenicol by 14 isolates while this was followed by a higher antimicrobial resistance of 66.67% against Augumentin by 5 isolates. The lowest antimicrobial resistance of 6.67% was obtained for 1 isolate against Ciprofloxacin (table 4).

Table.1 Average bacterial count of isolates from the samples

LOCATION	CODE	MCA WITH STANDARD ERROR
EDE SAWMILL	ESS	$8.2 \times 10^6 \pm 20.43$
	ESW	$5.0 \times 10^6 \pm 5.77$
	ESF	$1.35 \times 10^7 \pm 20.82$
	ESG	$1.40 \times 10^7 \pm 7.94$
EDE MOBI	EMS	$2.92 \times 10^7 \pm 5.13$
	EMW	$1.81 \times 10^7 \pm 18.01$
	EMF	$9.5 \times 10^5 \pm 18.93$
	EMG	$1.00 \times 10^7 \pm 17.56$
OSOGBO RING ROAD	ORS	$2.27 \times 10^7 \pm 22.86$
	ORW	$1.46 \times 10^7 \pm 17.06$
	ORF	$1.60 \times 10^7 \pm 10.41$
	ORG	$9.1 \times 10^6 \pm 10.41$

Key:

ESS –Ede Sawmill Sediment
ESF – Ede Sawmill Feed
ESW – Ede Sawmill Water
ESG – Ede Sawmill Gut
EMF – Ede Mobi Feed
EMW – Ede Mobi Water
EMS – Ede Mobi Sediment
EMG – Ede Mobi Gut
ORS – Osogbo Ringroad Sediment
ORG – Osogbo Ringroad Gut
ORW – Osogbo Ringroad Water
ORF – Osogbo Ringroad Feed

Table.2 Percentage bacteria in different locations

Location	Ring road	Mobi	Sawmill
Pond water	4(15.38%)	8(30.77%)	2(18.18%)
Water sediment	2(18.18%)	9(34.62%)	3(27.27%)
Fish gut	3(27.27%)	5(19.23%)	3(27.27%)
Fish feed	2(18.18%)	4(15.38%)	3(27.27%)

Table.3 Bacteria distribution in each sample

ISOLATES	POND WATER	FISH FEED	POND SEDIMENT	FISH GUT	TOTAL OCCURENCE IN THE SAMPLES
Escherichia coli	1	2	1	3	7(14.58%)
Aeromonas species	1	1	1	2	5(10.41%)
Salmonella species	1	2	2	-	5(10.41%)
Proteus species	2	-	2	-	4(8.33%)
Citrobacter freundii	2	-	1	-	3(6.25%)
Morganella morganii	2	1	2	-	5(10.41%)
Enterobacter species	1	-	1	2	4(8.33%)
Serratia species	1	1	1	1	4(8.33%)
Klebsiella species	2	1	2	-	5(10.42%)
Provindencia species	1	-	1	1	3(6.25%)
Shigella species	-	1	-	2	3(6.25%)

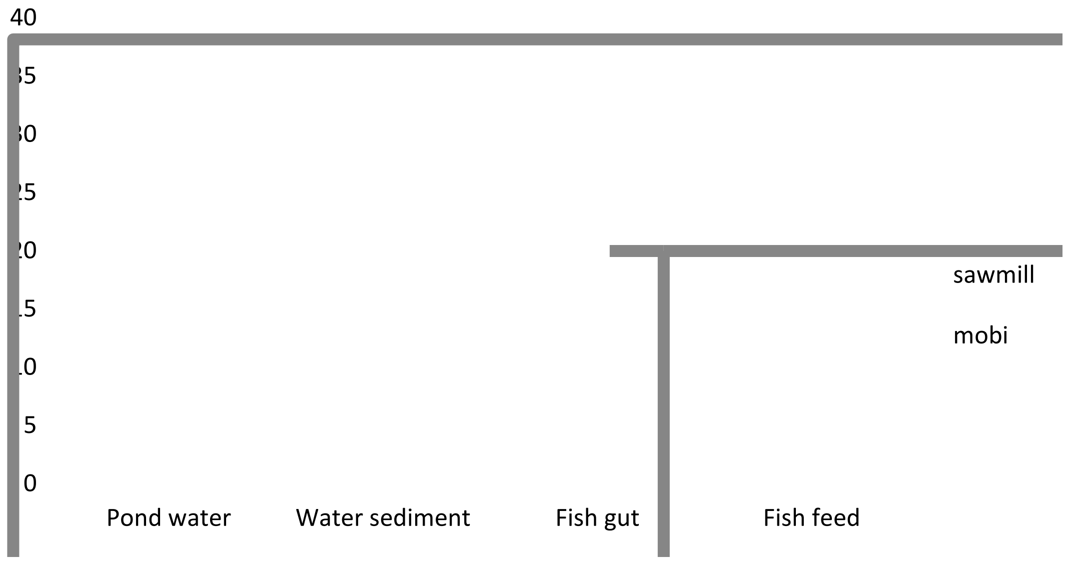
Table.4 Percentage susceptibility pattern of bacteria to antibiotics

Antibiotics	Sensitivity S (%)	I	Resistivity R (%)
SXT	1 (6.67%)		14 (93.33%)
CH	1 (6.67%)		14 (93.33%)
CPX	14 (93.33%)		1 (6.67%)
AM	7 (46.67%)		8 (53.3%)
AU	5 (33.33%)		10 (66.67%)
GN	10 (66.67%)		5 (33.33%)
PEF	10 (66.67%)		5 (33.33%)
OFX	9 (60%)		6 (40%)
S	8 (53.33)%		7 (46.67%)
SP	13 (86.67%)		2 (13.33)

Key

SXT = Septrin	CH = Chloramphenicol
CPX = Ciprofloxacin	AM = Amoxacilin
AU = Augmetin	GN = Gentamycin
PEF = Perfloxacin	OFX = Tarivid
S = Streptomycin	SP = Sparfloxacin
R = resistant	S = sensitive

Figure.1 Percentage (%) of bacteria isolates per location



The isolation of Enterobacteriaceae: *Escherichia coli*, *Aeromonas species*, *Salmonella species*, *Proteus species*, *Citrobacter freundii*, *Morganella morganii*, *Enterobacter species*, *Serratia species*, *Klebsiella species* ,*Provindencia species* and *Shigella species* from the three locations are in line with the findings of Ogbondeminu and Olayemi (1993) who reported that 50% of the microorganisms recovered from both fish and water of earthen pond fertilized with animal faecal waste were members of the family *Enterobacteriaceae*.

The presence of *E*. coli in all the samples indicated poor hygiene and sanitary condition in all the locations. This is in line with the study of Chandraval *et al.,* (2010) which reported that fish and water samples collected from Nadia District of West Bengal in India were contaminated with faecal coliforms.

Aeromonas species, one of the bacteria isolated from the samples is a known human pathogen which poses a risk of fish-borne *Aeromonas gastroenteritis* in consumers of improperly cooked fish however this has shown that consumption of antibiotic in fishes has become a serious issue in various environments for human health concern (Neela *et al.,* 2012; Rahman *et al.,* 2008) and it has been well demonstrated that aquatic environments play a vital role for spreading of antibiotic resistant bacteria and gene in various ecosystem (Neela *et al.,* 2009).

Surplus antimicrobial agents accumulate in integrated fish farms when the ponds are rarely emptied at the time of fish harvest, such an accumulation has been reported to establish selective pressure favoring selection and growth of antimicrobial resistant bacteria (Andreas *et al.,* 2002). This may be the reason why some of the bacteria isolates used for this study were able to resist all the tested antibiotics.

Potential transfer of resistant bacteria and resistance gene from aquaculture environment to human can occur through direct consumption of antimicrobial resistant bacteria present in fish and associated products and this can lead to an increase in the number of infections, an increased frequency of treatment failure and increased severity of infection (Andreas *et al.,* 2002)

Conclusion of the study is as follows:

In view of the isolation of potential bacterial pathogens from all the sample types and high level of multi drug resistant bacterial contamination reported in this study necessary measures must be put in place to prevent the release or discharge of antimicrobial residues and antimicrobial resistant bacteria into ponds in Nigeria, particularly in Osun state in order to prevent the transfer of resistant genes from fish pathogens to human as the effect can be devastating if consumed by man. Such measures include: discouragement of the indiscriminate use of antimicrobial agents in integrated fish farms, discouragement of the use of faeces of poultry and encouragement of the use of feeds with high microbial quality in order to avoid using antimicrobials as growth additives. There should also be regular drainage of pond water after specific period of time. In addition to the above, there should equally be public enlightenment programme on the inherent danger that may accompany the consumption of improperly cooked fish.

Lastly more elaborate studies especially multicenter studies should be carried out to determine the impact of Integrated Fish Farming on Antimicrobial Resistant Bacteria in ponds on a national level in order to enable formulation of guidelines for monitoring and establishing preventive programmes.

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