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Pengaruh antibiotik terhadap keragaman mikrobioma usus pada ikan *Clarias gariepinus* dari pembenihan komersial

Effect of antibiotics on gut microbiome diversity in *Clarias gariepinus* from commercial hatcheries

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Abstract

The study investigated the effects of antibiotics on gut microbiome diversity in fish (*Clarias gariepinus*). Total Aerobic Count (TAC), Total Coliform Count (TCC), and Total Fungal Count (TFC) of gut contents were determined. Results revealed that there were significant differences ($p<0.05$) observed in the total aerobic and total coliform counts across the hatcheries. Using the Shannon and Simpson indices to measure variability in bacterial diversity among the hatcheries, TAC in hatcheries using antibiotics had greater diversity compared to hatcheries not using antibiotics while TCC where hatcheries not using antibiotics had higher diversity than the ones using antibiotics. This indicates that hatcheries where antibiotics were not used had higher microbial densities than hatcheries where antibiotics were used. However, it was observed that fungi were completely absent as indicated in the total fungal counts. In hatcheries using antibiotics, *Shigella* spp, *Klebsiella* spp, and *Salmonella* spp were isolated while in hatcheries not using antibiotics, *Shigella* spp, *Klebsiella* spp, and *Enterobacter* spp were isolated. Sorenson's coefficient indicated a slight overlap or similarity with a value of 0.667 among the bacterial communities in samples from farms using antibiotics and ones not using antibiotics. All the bacteria isolated belong to the phylum Proteobacteria. The study revealed that antibiotics had a slight effect on the diversity of the fish gut microbiome indicating that antibiotics are moderately used in fish hatcheries in Ijebu-Ode Region of Ogun State.

Keywords: Antibiotics; Diversity; Gut Microbiome

1. Introduction

Globally, aquaculture is the fastest food producing industry (FAO, 2016). Due to high intensity of production and its associated bottlenecks, aquaculture production in Nigeria is grossly characterised by unregulated use of antimicrobial agents. They are often used by fish farmers for treatment and control of diseases in fish (Durojaiye and Sule, 2018). Antibiotics administration is intended to help fish fight infections. However, it can have a detrimental effect on the commensal intestinal microbial communities of the host. The gut of fish harbours a diverse and complex communities of microorganisms known as gut microbiome or microflora. The beneficial roles of the gut microbiome include stimulation of nutrient metabolism, innate immune response and epithelial proliferation (Roeselers *et al*., 2011). Additionally, gut microbiome constrains the colonization of infection agents and, by interacting with the host, mediate the development, maintenance and effective functionality of the intestinal mucosa (Giatsis, 2016; Feng *et al*., 2018). Hence, maintaining a normal gut microbiome diversity is essential for the growth of the aquaculture industry.

The composition of the gut microbiome in fish is greatly influenced by diet and its environment (Li *et al*., 2012; Shahdat *et al.,* 2014).). Since antibiotics kill or inhibit the growth of bacteria (Cabello *et al*., 2013), this may result in a reduction in bacterial diversity, thereby causing a change in the presumably beneficial host-micro biota relationship. Hence, possible modifications in the gut microbiome of farmed fish due to antibiotic treatment could arise. And in worst case scenario, it can result in eradication of the normal gut microbiome, hence facilitating the proliferation of opportunistic bacteria by depleting competition. Therefore, it is imperative that the effects of antibiotics on fish gut microbiome be investigated.

Very few studies have focused on determining the effects of antibiotics treatments on the microbial ecology of the fish gut. In general, studies have mainly focused on describing the frequency of antibiotic resistance during and after the use of antibiotics (Han *et al*., 2020), the susceptibility of fish pathogens isolated from fish and fish farms to antibiotics (Durojaiye *et al*., 2023) and molecular determinants of antibiotic resistance (Estruch *et al*., 2015). Previous study by Durojaiye *et*

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al., (2020) have established the unregulated use of antibiotics in fish hatcheries in Ijebu-Ode Region of Ogun State. However, the studies did not investigate the effects of antibiotics on fish intestinal microbial diversity. Against this background, this research looked into the effects of antibiotics administration on the gut microbiome of *Clarias gariepinus* from fish hatcheries in Ijebu-Ode, Ogun State, Nigeria.

2. Materials and Methods

2.1. Study area

The study was carried out in Ijebu-Ode Local Government of Ogun State, Nigeria. Ijebu-Ode is a city located in South-Western Nigeria at latitude 6.8944 and longitude 3.911731. Fish farming is well established and is one of the major or secondary agriculture-related occupation in the area.

2.2. Sample collection

A total of 250 *C. gariepinus* fingerlings were collected from five different fish hatcheries in Ijebu Ode. They were assigned into 5 groups with 50 fingerlings each and labelled as follow:

AA – Farm A using antibiotics (Floxinor)
AB – Farm B using antibiotics (Tetracycline, Floxinor, Potassium permanganate)
AC – Farm C using antibiotics (Potassium permanganate, Procaine penicillin)
NAA – Farm A not using antibiotics
NAB – Farm B not using antibiotics

2.3. Sample preparation

Fish samples were dissected aseptically underbelly to remove the intestines. The collected intestines were kept in labelled containers. The media used (nutrient agar, manittol salt agar, *Salmonella-shigella* agar, euosin methylene blue agar and rose Bengal chloramphenicol agar (rBca) were prepared according to manufacturers' prescription. The desired quantities were weighed into conical flasks with the corresponding quantity of distilled water, plugged with cotton wool wrapped in foil paper and homogenized in water bath. It was sterilized at 121^{o}C for 15 mins in an autoclave with the exception of *Salmonella-shigella* agar that was boiled and cooled.

2.4. Serial dilution

A set of five test tubes with cover were filled each with 9mls of distilled water and sterilized in an autoclave at 121^{0}C for 15mins.The tubes were labelled 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5} respectively. 1g of each of the fish samples were weighed into the tube labelled 10^{1}. Thereafter, the tube was vortexed and 1ml was pipetted from it into the tube labelled 10^{-2}. From this, 10^{-2} tube, 1ml was pipetted into tube labelled 10^{-3}. The process was repeated until the tube labelled 10^{-5} was done. From the tubes labelled 10^{-3} and 10^{-5}, 1ml each was pipetted ascetically into each petri dish and prepared molten but cooled agar was poured and swiped gently to allow for even mixing of the sample into the medium. Upon solidifying, the plates were inverted and incubated at 37^{0}C for 18-24hrs and the result read. However, yeast extract agar plates and rBca were incubated at room temperature for 2-5days. Colonies were counted from each plate and recorded. The district colonies were sub-cultured, gram stained, and series of biochemical tests carried out to identify the isolated organisms.

2.4. Data analysis

The data obtained were analyzed using two-way analysis of variance SPSS version 23.0 software package and results presented as means ± standard deviation. Significance among groups was tested using Duncan's Multiple Range test at p=0.05. Sorenson's coefficient, Shannon Index and Simpson Index were also used to measure similarity and diversity of the gut microbiome across the hatcheries (antibiotics/ no antibiotics) using the following equations:

$$\text{Sorenson's coefficient} = \frac{2C}{S1-S2}$$

c- number of species the community have in common
S1- total number of species in community 1 (antibiotics)
S2- total number of species in community 2 (no antibiotics)

$$\text{Shannon Index (H)} = \sum_{i=1}^{s} \text{piInpi}$$

$$\text{Simpson Index (D)} = \frac{1}{\sum_{i=1}^{s} \text{pi}^2}$$

3. Results and Discussion

3.1. Results

Table 1 shows the mean variations of gut microbiome population densities in fish from various hatcheries. There were significant differences ($p<0.05$) observed in the total aerobic and total coliform counts across the hatcheries. NAA and NAB recorded the highest values for TAC and TCC while the least counts were recorded in AA.

Table 1
Mean variation in Microbial load treated with antibiotics and non-antibiotics in fish samples (cfu x10^{5}).

Farm	TAC	TCC	TFC
AA	1.10 ± 0.10^{c}	0.00 ± 0.00^{d}	
AB	1.45 ± 0.08^{b}	1.167 ± 0.08^{b}	
AC	1.35 ± 0.15^{b}	0.75 ± 0.14^{c}	NG
NAA	1.71 ± 0.09^{a}	1.46 ± 0.06^{a}	
NAB	1.75 ± 0.26^{a}	1.32 ± 0.55^{a}	

- Mean ± S.D with different alphabets showed significant differences ($p<0.05$)
- TAC- Total Aerobic count, TCC- Total coliform count, TFC- Total fungal count, CFU- Colony forming unit, NG- No growth

In Table 2, the Shannon and Simpson indices showed variability in bacterial diversity among the hatcheries. The results indicated that TAC in hatcheries using antibiotics, had greater diversity compared to hatcheries not using antibiotics. However, the opposite was observed in TCC where hatcheries not using antibiotics had higher diversity than the ones using antibiotics.

Table 2
Shannon and Simpson Indices for bacterial diversity.

Treatment	Shannon/Simpson Index	TAC	TCC
Antibiotics	H	2.183	1.338
	D	3.012	1.908
No antibiotics	H	1.386	1.384
	D	2.00	1.99

As presented in Table 3, the diversity of the bacteria isolated and identified from the fish gut was low. In hatcheries using antibiotics, *Shigella spp*, *Klebsiella spp* and S*almonella spp* were isolated while in hatcheries not using antibiotics, *Shigella spp*, *Klebsiella spp* and *Enterobacter spp* were isolated with *Klebsiella spp* occurring in all farm locations. Using the Sorenson's coefficient, the bacterial communities (antibiotics/no antibiotics) have a slight overlap or similarity with a value of 0.667.

Table 3
Diversity of bacteria isolated from fish gut.

Farm	Isolated Organism(S)	Phylum	Group	Gram (+/-)
AA	*Shigella spp*	Proteobacteria	Facultative anaerobe	Gram -
	Klebsiella spp	Proteobacteria	Aerobe/facultative anaerobe	
AB	*Salmonella spp*	Proteobacteria	Facultative anaerobe	Gram -
	Klebsiella spp	Proteobacteria	Aerobe/facultative anaerobe	
AC	*Salmonella spp*	Proteobacteria	Facultative anaerobe	Gram
	Klebsiella spp	Proteobacteria	Aerobe/facultative anaerobe	
NAA	*Shigella spp*	Proteobacteria	Facultative anaerobe	Gram
	Klebsiella spp	Proteobacteria	Aerobe/facultative anaerobe	
NAB	*Enterobacter spp*	Proteobacteria	Facultative anaerobe	Gram -
	Klebsiella spp	Proteobacteria	Aerobe/facultative anaerobe	

3.2. Discussion

Diseases in fish usually occur under stress conditions resulting in high mortalities and significant economic loss (Sudheesh *et al*., 2012). To avoid such huge losses, fish farmers use antibiotics and other antimicrobial agents mostly for the prevention and treatment of diseases in fish (Pham *et al*., 2015; Durojaiye *et al*., 2019). They are also used to ensure good water quality and disinfect eggs and equipment (Cabello *et al*., 2013; Durojaiye and Sule, 2018). Antibiotics kill or inhibit the growth of bacteria (Cabello *et al*., 2013), hence, may have an effect on fish gut microbiome population density. In this study, the hatcheries where antibiotics were not used had higher microbial densities than hatcheries where antibiotics were used. However, it was observed that fungi were completely absent as indicated in the total fungal counts. The reason for this might be because the fish gut is naturally dominated by bacteria (Rombout *et al*., 2011; Ghanbari *et al*., 2015).

Clements *et al*. (2014) and Estruch *et al*. (2015), opined that gut microbiome of herbivorous animals has the greatest diversity, and that this diversity would decrease among omnivores and decrease further among carnivores. The low diversity recorded in this study could be attributed to the omnivorous feeding nature of the fish sampled.

All the bacteria isolated in this study belong to the phylum Proteobacteria. This corroborates earlier studies on fish gut microbiome that Proteobacteria is the most dominant bacterial phylum in fish gut. In addition, the bacteria isolated are either facultative anaerobe or aerobes (Lozupone *et al*., 2012; Clements *et al*., 2014). This also agrees with the findings of Estruch *et al*. (2015) who reported that dominant bacteria are typically either aerobes or facultative anaerobes. However, some studies have documented obligate anaerobes as part of the gut microbial assemblage in fish gut. According to Zhou *et al*. (2021), the bacterial communities in the fish intestine were dominated by Gram-negative aerobes. This report aligns with the findings from this study where 100 percent Gram negative aerobes or facultative anaerobes were recorded.

4. Conclusion

The study revealed that antibiotics had a slight effect on the diversity of the fish gut microbiome indicating that antibiotics are moderately used in fish hatcheries in Ijebu-Ode Region of Ogun State. However, continuous monitoring and awareness campaign should be employed in order not to abuse the use of antibiotics in fish production.

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