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Original Research Article

Occurrence of haemolytic *Escherichia coli*, antimicrobials residue in cultured *Clarias gariepinus* and assessment of antimicrobial use among catfish farmers in Kano metropolis, Nigeria

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ABSTRACT

Antibiotics have been utilized as both antibacterials and growth-promoting agents, although their residues have been reported to be hazardous to both animals and humans. This study aimed to determine the presence of drug resistant haemolytic *Escherichia coli* in cultured African catfish, detect chloramphenicol and furaltadone residues in fish-fillets, and assess knowledge, attitude and practice of fish farmers on antimicrobial usage. Liver and fillets samples (N=400) from 10 commercial fish farms (n=40) were examined. Isolates were identified after Grams' staining using conventional biochemical tests. Antimicrobial susceptibility was tested using Kirby-Bauer disc diffusion technique and results were interpreted using clinical laboratory standard institute(CLSI) guide. Detection of drug residues was done using high performance liquid chromatography (HPLC). Antimicrobial use (knowledge, attitude and practice) of fish farmers were assessed usingsemi-structured questionnaire. Haemolytic *E. coli* (69.3) were isolated and 63 % were observed to be resistant to chloramphenicol, furaltadone, gentamicin, amoxicillin, erythromycin, tetracycline, penicillin, streptomycin, nitrofurantoin and doxycycline. Chloramphenicol and furaltadone residues were not detected in all the samples. Most fish farmers 18 (60%) lack knowledge of antimicrobial resistance and withdrawal period 22 (73.3 %). Their sources of information on antimicrobial usage are co-farmers, drug-vendors and internet. In this study from cultured *Clarias gariepinus* the occurrence of haemolytic *E. coli* was 69.3 % and that of chloramphenicol, furaltadone and their metabolites was zero. The *E. coli* isolated were resistant to at least 4 of the 10 antimicrobials tested.

Keywords: Antimicrobials, cultured-catfish, *Escheria coli*, HPLC, Kano-Nigeria.

INTRODUCTION

African catfish has been the most widely cultured and highly accepted fish species in Nigeria (FAO, 2017). Due to its high-quality dietary protein and low-fat content, catfish are relished as a delicacy among Nigerians for home consumption or at fast food joints and restaurants. The growing demand for catfish coupled with the quest for increased yield and profit has led to intensification and private investment in catfish production in Nigeria (FDF, 2007). However, diseases both infectious and non-infectious constitute a major constraint to the aquaculture productivity (Bagumire *et al.*, 2010).

Generally, bacterial diseases in fish do not occur as a result of exposure to infectious agent (Wedekind *et al.*, 2010). In most cases, disease occurs following complex interactions between fish and the pathogen under stressful environmental conditions (Song *et al.*, 2008). Although *Escherichia coli* (*E. coli*) is considered to be normal inhabitants of the

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gastrointestinal tracts, it causes severe outbreaks of disease with mortalities following environmental stress or injury (Wedekind *et al.,* 2010). Fish under intensive culture are usually exposed to extreme environmental fluctuations and therefore tend to be more sensitive to stress than wild fish populations (Salah *et al.,* 2012). Cultured fishes were throughout history not believed to be important vectors of human pathogens (Greenlees *et al.,* 1998). This narrative is however changing with increasing animal densities, consequent rapid growing of the aquaculture industry and partly due to increased awareness of pathogens from aquatic species that may result in human illness (Salah *et al.,* 2012).

Antimicrobials are being used as food additives, for prophylaxis and therapeutics (Omeiza *et al.,* 2012). Irrational use of these antimicrobials for prophylaxis or as additives may increase the risk of the pathogenic bacteria becoming resistant (Maciej *et al.,* 2020).

The world health authorities have discouraged the use of chloramphenicol and furaltadone in food-producing animals (Dinos *et al.,* 2016). In view of this development, a zero tolerance level was indicated for their presence as residues in foods of animal origin destined for human consumption (Lynas *et al.,* 1998). In Nigeria, many fish farmers use chloramphenicol to control fish diseases because of its claimed efficacy (Omeiza *et al.,* 2012). In some situations, farmers were seen adopting chloramphenicol dosage forms meant for humans for veterinary use. Okonko & Ogbonna (2018) reported that farmers in Nigeria still use furaltadone in the form Agrar fural ®, Furasol ® and Agra-cox ® preparation for treatments. However, their use in Veterinary Medicine has been banned due to concerns about the carcinogenic tendency of their residues in food animal tissues (Vass *et al.,* 2008). Excessive and inappropriate antimicrobial use play a great role in the development of resistance. Wrong diagnosis, knowledge, belief, expectations and attitudes of farmers towards antimicrobials are also responsible for increased emergence and spread of antibiotic resistant microorganisms (Hulscher *et al.,* 2010).

There is paucity of data on antimicrobial residues and usage in fish in Nigeria. Therefore, studies that determine and quantify the contribution of aquaculture and aquatic environments on the appearance of infections by antibiotic resistant bacteria are essential (Sérgio *et al.,* 2018). This study aimed to determine the occurrence of drug resistant haemolytic *E. coli,* chloramphenicol and furaltadone residues in cultured *Clarias gariepinus* and assessed antimicrobial usage among fish farmers in Kano Metropolis, Nigeria.

MATERIALS AND METHODS

STUDY AREA

Kano Metropolis is located at the Central Western part of Kano State between latitude 11059´59.57 – 12002´39.57^{0}N of the equator and between longitudes 8033´19.69 – 8031´59.69^{0}E. It lies in the Northern Central boundary of Nigeria and is located some 840 km away from the edge of the Sahara Desert and 1,140 km from the Atlantic Ocean (Oseiki, 2009). Kano State is located in the semi-arid zone of Northern Nigeria and enjoys the warm tropical climatic condition of Western Africa. Most parts of Kano State fall within the Sudan savannah vegetation zone, whereas the far southern area falls within the Guinea vegetation zone. However, within the two major types of zones identified, trace of other vegetation also exists. Its metropolis population is the second largest in Nigeria, after Lagos. Kano state has a mean height of about 472.5 m above sea level. Kano city has expanded over the years and has become the third largest in Nigeria. The Kano Urban area covers 137 km² and comprises eight Local Government Areas (LGAs) (KSMI, 2005). The specific areas for the study included Municipal, Gwale, Dala, Tarauni, Nassarawa, Fagge, Ungogo, Kumbotso and Kano municipal LGAs.

STUDY DESIGN

Forty (40) samples were randomly drawn from 10 farms within the eight local government areas located in Kano metropolis, Nigeria. From each pond in a farm representative samples were obtained. Bacterial isolation, biochemical characterization, haemolysis and susceptibility tests were carried out and results interpreted. For the Chloramphenicol, furaltadone and or their metabolites detection ten samples each were pooled to make one sample for convenience, making a total of forty samples (four per farm). Semi-structured questionnaire was adopted to assessed knowledge, attitude and practice of the fish farmers towards antimicrobial use.

Data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 20 and the results were presented using tables, simple percentages (descriptive statistics) and chromatogram. Resistance of *E. coli* to antimicrobial agents was expressed as percentage. Responses of fish farmers to questionnaire were expressed as bar charts.

ETHICAL CLEARANCE

Approval was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2021/046).

DRUG REFERENCE STANDARDS

The standard powders were obtained from Yuane Biotechnology Company Limited located at No. 465

Changta road, Songjiang District, Shanghai China through Adels scientific and medical supplies.

SAMPLE COLLECTION

Catfish samples (n= 40 per farm) were obtained from 10 registered fish farms (N= 400) in Kano metropolis, Nigeria. All the farms operate pond fish culture, raising only African catfish. Fish sampled were between 22 to 25 weeks of age, had an average weight of 800 ± 79.16 g and average length of 32 ± 2.67 cm respectively. Fish samples were immobilized, incised longitudinally using a sterile scissors from the anal opening to the operculum. Liver and fillet samples were aseptically collected, labelled separately in small sterile nylon and transported in an iced container to Diagnostic Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine and Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria for the analysis.

ISOLATION AND IDENTIFICATION OF HAEMOLYTIC *E. COLI* FROM *CLARIAS GARIEPINUS* LIVER

Isolation and identification were carried out based on the procedure described by Quinn *et al.* (2002). Each sample was cultured on MacConkey agar and aerobically incubated at 37 ºC for 24 h. Briefly, using a hot sterile spatula the surface of the liver sample was seared. Sterilized scissor was then used to cut deep through the seared surface. Primary smear was made from the deep cut using a swab stick. Sterilized wire loop was then used to make secondary and tertiary streaks. The growth was sub-cultured on Eosin methylene blue (EMB) agar and aerobically incubated at 37 °C for 24 h. Colonial morphology was studied and the microbial growth was subjected to Gram staining and basic biochemical tests for identification in accordance with standard methods (Quinn *et al.,* 2002).The biochemical tests carried out were triple sugar iron (TSI), Indole, Urea, Citrate, MRVP (Methyl red Voges Proskeur) and motility. Isolates were sub-cultured on blood agar (Oxoid®CM0055 from UK) for haemolysis (pathogenicity testing). The isolates were preserved in a nutrient agar slant for antimicrobial susceptibility testing.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Susceptibility of the 277*E. coli* isolates to ten antimicrobials was tested using Kirby-Bauer single disc diffusion method Bauer *et al.*,(1966) and results interpreted using CLSI (2018) guide. A total of 10 antimicribials, 9 from Oxoid® containing chloramphenicol (30 mg), gentamycin (10 mg), amoxicillin (10 mg), erythromycin (15 mg), nitrofurantoin (50 mg), tetracycline (30 mg), penicillin (10 units), streptomycin (10 mg) and doxycycline (30 mg) were used and 1 furaltadone (50 mg) from Yuane Biotechnology Company Limited were tested. The antibiotics impregnated discs were applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto Mueller Hinton agar to ensure complete contact with the agar surface. The plates were inverted and incubated at 37 °C. After 24 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to CLSI (2018) interpretation guide.

ANTIMICROBIALS RESIDUE DETECTION

A modified form of the method described by Yiqing *et al.* (2013) was adopted throughout the HPLC residue detection procedures (pretreatment, sample extraction, preparation of stock and working solutions, method development and sample analysis).

FILLET SAMPLES PRETREATMENT

Samples were pooled 10 in 1 for this study. A portion (0.5 g) from each of the tissue samples was taken to make 5 g, this was subjected to dissolution in 5 g sodium sulfate and 10 mL ethyl acetate and centrifuged at 3000 revolutions min^{-1} for 6 minutes. The supernatant (A) was drained into 20 mL test tube. Another 10 mL portion of ethyl acetate was added to the residue, homogenized and further centrifuged at 3000 revolution min^{-1} for 20 minutes. The supernatant (B) was drained into a test tube and a portion (5 mL) was taken and added to 5 mL of supernatant A to form a combined extract (10 mL) which was air dried for proper storage.

SAMPLE EXTRACTION

A 10 mL methanol was later added to dissolve the dried residue and centrifuge at 3000 revolution min^{-1} for 10 minutes, 3 mL of the supernatant was then collected degreased thoroughly (mixed with 5 mL cyclohexane and centrifuged at 3000 revolution min^{-1} for another 10 minutes) to remove fats and other impurities, 2 mL of the supernatant was then collected for the analysis.

SAMPLE ANALYSIS FOR THE DETECTION OF CHLORAMPHENICOL AND FURALTADONE RESIDUES

The column retention time for the drugs reference standard was obtained on a Chemisil Octadecyl-silica (ODS) C18 column using an Agilent HPLC system consisting of an Agilent HPLC pump, an automatic injector fitted with a 20 µL sample loop at a wave length of 280 nm. Fourty (40) samples were analysed for presence of the antimicrobials residues.

ASSESSMENT OF ANTIMICROBIAL USE AMONG COMMERCIAL FISH FARMERS

Semi-structured questionnaire was randomly administered to thirty (30) aqua culturists within Kano Metropolis based on respondents availability. The questionnaire comprised sections on their knowledge, attitudes and practices as regards antibiotic usage and withdrawal period in their fishes, also knowledge of antibiotic resistance and residues. A pre-test of the questionnaire exercise was carried out with four catfish producers for a better understanding and clarity of the questions. The. Knowledge of antimicrobial resistance was investigated through practices of antibiotic usage involving indications, prescription, sources of information on antimicrobial use, administration, observance or non-observance of withdrawal period, sales and or consumption of fish under recent medications.

DATA ANALYSIS

Data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 20 and the results are presented using tables, simple percentages (descriptive statistics) and chromatogram.

Occurrence and susceptibility of *E. coli* to antimicrobial agents was expressed as percentage. Responses of fish farmers to questionnaire were expressed as bar charts using excel 2013.

RESULTS

CHARACTERISTICS OF THE *E. COLI* ISOLATED FROM CULTURED *CLARIAS GARIEPINUS*

Table I are the results of biochemical tests for identification of *E. coli* isolates. Table II shows the *E. coli* isolated on Eosin Methylene Blue (EMB) agar. Isolates were found in 2-3 mm diameter colonies characterized by greenish metallic sheen appearance in reflected light. The haemolytic patterns observed among the *E. coli* isolates were Alpha (59; 21.3 %), Beta (113; 40.79 %) and Gamma (105; 37.91 %) (Table III).

ANTIMICROBIAL SUSCEPTIBILITY OF HAEMOLYTIC *E. COLI* ISOLATED FROM CULTURED *CLARIAS GARIEPINUS*

The antibiogram revealed that 63 (63 %) of the *E. coli* isolated from the Cultured African catfish in the study area were resistant to at least 4 antimicrobial agents tested (Table III). Only 18 (18 %) and 19 (19 %) of the isolates were found to be susceptible and resistant, respectively, to the antimicrobial agents tested. Among the antimicrobials tested most isolates were resistants to Chloramphenicol (70 %), furaltadone (80 %), gentamicin (70 %), nitrofurantoin (80 %) and penicillin (90 %).

Table I: Biochemical tests on identification of *E coli* from the liver of *Clarias gariepinus*in Kano Metropolis, Nigeria.

Key: TSI = Triple sugar iron, K/A = Alkaline / Acid, A/A = Acid/Acid, + = positive, - = Negative

S/N	Test	*E. coli*
1.	Grams reaction	-
2.	Oxidase	-
3.	TSI	A/A
4.	Indole	+
5.	Methyl red	+
6.	Voges-proskauer	-
7.	Citrate	-
8.	Urease	-
9.	Motility	+

Table II: Frequency of Distribution of *E. coli* isolates from the liver of cultured African catfish (*Clarias gariepinus*) from 10 Commercial Farms in Kano Metropolis, Nigeria Total number of samples (N) =400, Number of samples per farm (n) = 40

S/No.	Farm	No. of *E. coli* isolates	Occurrence per farm (%)
i.	Allah gatan kowa	8	20
ii.	Dan Ahmad	34	85
iii.	*Dabino*	36	90
iv.	Jaba	24	60
v.	Ladanai	28	70
vi.	Nabarira	27	68
vii.	Rangaza	33	85
viii.	*Rumbun kifi*	34	83
ix.	Unnamed	32	80
x.	Zamani	21	53
Total		277	69.3

Table III: Haemolytic Pattern Observed in *E. coli* isolates from *Clarias gariepinus* in Kano Metropolis

S/N	Types of Haemolysis	Number	Percentage (%)
1.	Alpha (α)	59	21.30
2.	Beta (β)	113	40.79
3.	Gamma (γ)	105	37.91
	Total	277	100

Table IV: Assessment of Antimicrobial use among fish farmers in Kano Nigeria

S/N	**Questions**
1.	Do you know most of the antibiotics for fish use?
2.	Have you ever heard of antibiotic resistance?
3.	What do you think can cause antibiotic resistance?
4.	Are you aware that some Commercial fish feed contain antibiotic?
5.	Do you know that some antibiotics have been banned for use in food animals?
6.	If yes, can you name them?
7.	What is your sources of information on antibiotic use?
8.	Are you aware of drug withdrawal period?
9.	Do you agree that antibiotics can be used in feed to promote growth in fish?
10.	Do you agree that antibiotics can be used in feed to improve efficiency in fish?
11.	Is it good to give medication to fish even when there is no disease problem?
12.	How many times do you give fish medication before you sell them?
13.	What is your primary reason for using antibiotics on your farm?
14.	Which antimicrobial is in use now?
15.	Purpose of the antimicrobial usage?
16.	Was there prescription?
17.	Route of administration
18.	Duration of administration
19.	Withdrawal period
20.	Previous antimicrobial used (1, 2, 3 months or more ago)
21	Do you normally seek Veterinary advice before antibiotic use in fish?
22	What do you do if the drug you administered to your fish fail to stop the disease problem?
23	Do you have sometimes requested for antibiotic sensitivity test in any nearby Laboratory?
24	Do you engage veterinary services from time to time on your farm?
25	Do you sometimes ask Drug sellers/Retailers for advice?
26	Do you ask fellow farmers for advice on which drug to use?
27	Which are your most widely used drugs on the farm?
28	Which drugs give you the best results after use?
29	What drug combinations do you prefer to use each time you have disease problem in your fish?
30	Do you observe withdrawal periods after treatment before you fish?

DETECTION OF CHLORAMPHENICOL AND FURALTADONE RESIDUES IN *CLARIAS GARIEPINUS*

No residues or metabolites of the antimicrobials were detected from any of the samples despite repeatedly detecting the reference standards as shown by chromatograms in Figure I (Negative control), Figure II (Chloramphenicol reference standard), Figure III (reference standard for both chloramphenicol and furaltadone), FigureIV (representative sample).

RESPONSES OF FISH FARMERS ON ANTIMICROBIAL USE IN KANO METROPOLIS, NIGERIA

Table IV shows the questions answered by the respondents from the study area. Figures V, VI, VII, VIII and IX are the responses of the fish farmers to the questions on antimicrobial use. The farmers' responses also showed that the most common and widely used antimicrobial agents were: oxytetracycline, neomycin, erythromycin, streptomycin, florfenicol, furazolidone, chloramphenicol, potassium permanganate, malachite green, penicillin and table salt.

DISCUSSION

The colonial morphology, microscopic and biochemical characteristics of the *E. coli* recovered from cultured *Clarias gariepinus* in the study area are complementary and consistent with previous reports by Akande & Onyedibe (2019).

A higher prevalence (69.25 %) for *E. coli* was observed in catfish from this study when compared to the findings of previous reports is a source of concern. Akande & Onyedipe (2019) and Adanech *et al.* (2018) reported occurrence of17.5 % and 12 % in fish in Nigeria and Ethiopia, respectively. However, a higher prevalence (71 %) was observed in freshwater fish in China by Jiang *et al.* (2012). The relatively high prevalence of *E. coli* in African catfish in this study when compared to others, could have been influenced by the quality of the water source for aquaculture which may vary in the different studies. It may also signify that fish farmers in our study area pay little attention to hygienic practices in their source of water for aquaculture. Fish obtained directly from ponds are also likely to have a higher load of *E. coli* resulting from poor management, poor sanitary conditions in the farms and substandard hygiene practices associated with many artificial ponds especially in developing countries. The occurrence of *E. coli* in fresh fish as revealed by this study emphasizes that fresh fish could be a potential source of human infection, thus making this an issue of public health concern. The spread of such infectious agents to humans could occur not only by consumption of raw or

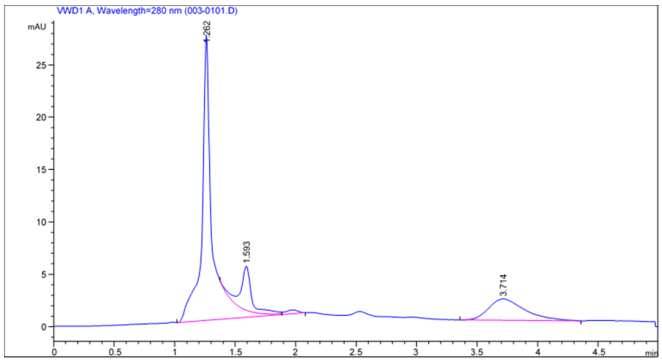

Figure I: Blank (methanol) Column Retention Time (1.262mns)

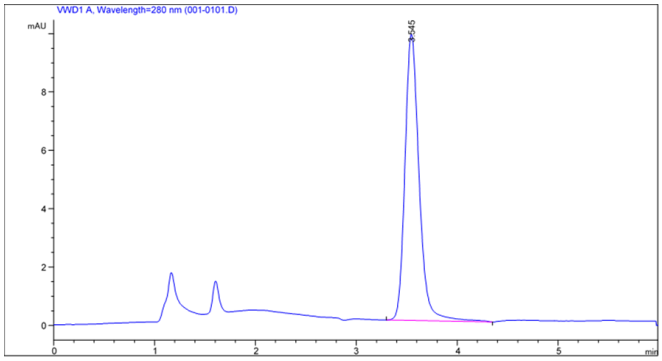

Figure I: Blank (methanol) Column Retention Time (1.262mns)

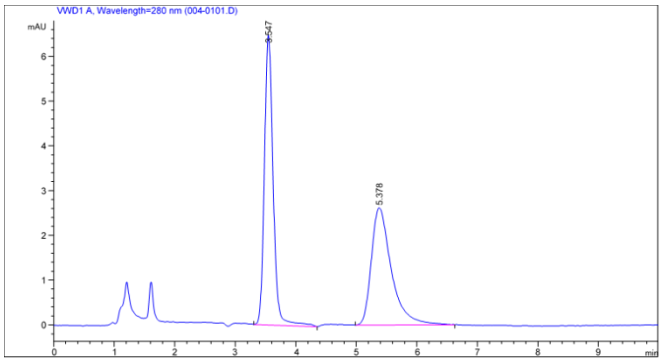

Figure III: Chloramphenicol & Furaltadone Reference Standard HPLC Column Retention Time (3.549 & 5.354 mns respectively)

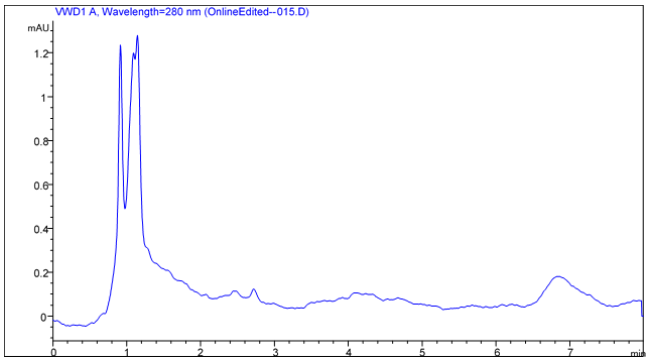

Figure IV. Chromatogram of result from a sample

undercooked fish, but also by environmental spread during handling.

It is important to note that haemolytic *E. coli* isolated from the liver of the African catfish in this study may seriously compromised the fish health. This assertion might be supported by the work of Akande & Onyedibe (2019) who reported significantly reduced packed cell volume and higher percentage of red blood cell lysis in experimentally infected African catfish.

The finding that 63 % of the *E. coli* isolates were resistant to at least 4 drugs tested could have far-reaching effects on public health because, with the emergence of antimicrobial

The observation that no residues of chloramphenicol and furaltadone were detected in the fillets of the African catfish sampled is consistent with previous reports by Reda *et al.* (2013) and Mensah*et al.* (2019). This finding is encouraging and is in compliance with international food standards of zero tolerance recommended for the two drugs by the FAO and WHO Lepretre and Merten-Lentz (2018).

Although their residues were not detected in the fillets of cultured catfish used in this study, the high resistance to chloramphenicol and furaltadone exhibited by *E. coli* might be of serious concern because these drugs are still considered among the less costly and most widely used for the treatment of some livestock as well as some human diseases as reported by (Wakawa *et al.*, 2015; Akande & Onyedibe, 2019). However, it may be pertinent to say that the findings in this study implies that fish farmers in the study area did not use the two banned drugs.

It has also been reported that there is no safe level of residues of chloramphenicol and furaltadone or their metabolites in food that represents an acceptable risk to consumers.

This may be explained by the fact that the reference drugs for both chloramphenicol and furaltadone were repeatedly detected by the technique during the assay for the two drugs. Also, the technique might have readily detected furaltadone if administered at all to the catfish in the farms, given that the depletion half-lives of the drug and its protein-bound metabolites were reported to be 15 days and 42 days, respectively, after cessation of treatment (Cooper *et al.*, 2006).

Majority of the fish farmers use drugs indiscriminately without veterinary prescription. This could have a far-reaching effects on both animal and human health. It is important to note that indiscriminate use of drugs in fish could lead to undesirable deposition of their residues in edible tissues offered for human consumption which could pose public health risks to the consumers. Associated public health risks include acute or cumulative allergic, toxic, mutagenic, teratogenic or carcinogenic effects. Antibiotic residues transferred to humans through food can also alter

Table IV: Antimicrobial Susceptibility of *Escherichia coli* isolated from cultured African catfish (*Clarias gariepinus*) in commercial farms in Kano metropolis, Nigeria

S/N	Antimicrobial	Concentration (mg)	Susceptible (%)	Intermediate (%)	Resistant (%)	Total
1	Chloramphenicol	30	2 (20)	1 (10)	7 (70)	10
2	Furaltadone	50	2 (20)	0	8 (80)	10
3	Gentamicin	10	2 (20)	1 (10)	7 (70)	10
4	Amoxicillin	10	2 (20)	3 (30)	5 (50)	10
5	Erythromycin	15	3 (30)	2 (20)	5 (50)	10
6	Nitrofurantoin	50	0	2 (20)	8 (80)	10
7	Tetracycline	30	2 (20)	3 (30)	5 (50)	10
8	*Penicillin	10 units	0	1 (10)	9 (90)	10
9	Streptomycin	10	3 (30)	3 (30)	4 (40)	10
10	Doxycyclin	30	2 (20)	3 (30)	5 (50)	10
	Total		18 (18)	19 (19)	63 (63)	100

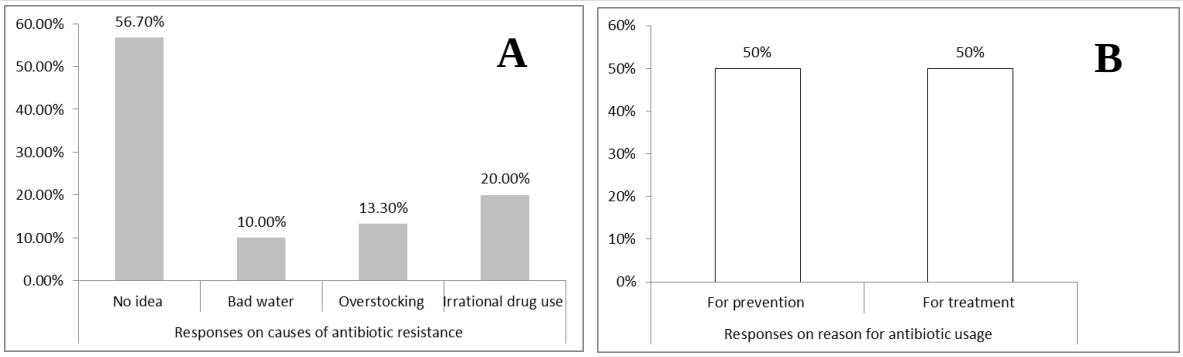

Figure V: Responses of fish farmers in Kano metropolis on antimicrobial resistance (A) and use (B)

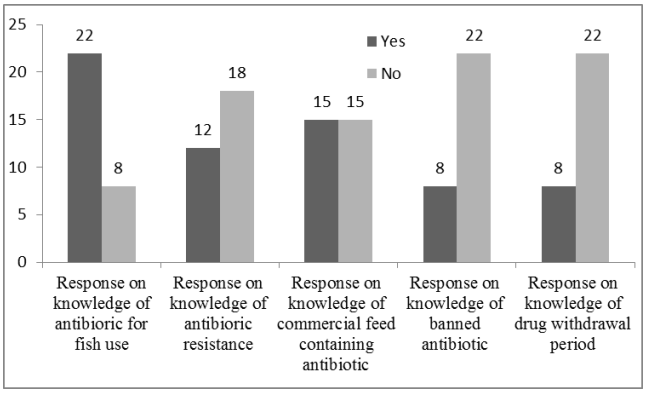

Figure VI: Responses of Fish Farmers on Antimicrobial Use in Kano Metropolis, Nigeria

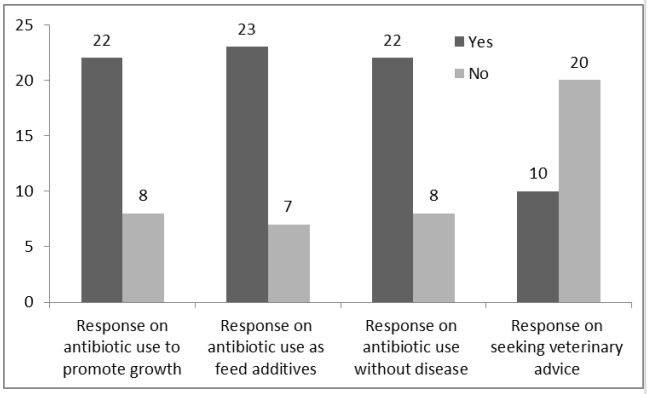

Figure VII: Responses of Fish Farmers on Antimicrobial Use in Kano Metropolis, Nigeria- Reason for use

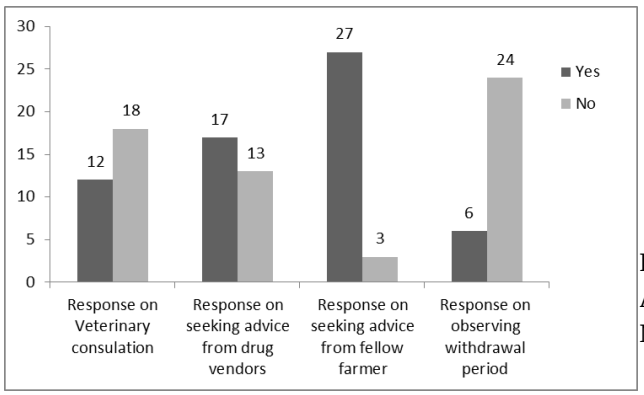

Figure VIII: Responses of Fish Farmers on Antimicrobial Use in Kano Metropolis, Nigeria- Source of advice

the intestinal ecology thereby favouring the emergence of resistant microflora (Olatoye & Basiru 2013; Moshina *et al.* 2016). More worrisome is the fact that most of the drugs tested are of medical importance to humans, as such the indiscriminate use of the drugs by the fish farmers might results in other side effects of antimicrobial residue in humans including aplastic anaemia with chloramphenicol, damage to urinary vestibular and auditory functions by aminoglycoside antibiotics, hypersensitivity reactions in human by penicillin (Olatoye & Basiru, 2013). Toxic and allergic reactions in humans and animals caused by oxytetracycline have only been observed at therapeutic dose. Oxytetracycline has been reported to produce immunosuppression in some fish species including human pathogens (Wakawa *et al.*, 2015).

From their responses, the finding that some fish farmers still use malachite green as a fungicide in fish, despite its banned, is worrisome. This is in view of the fact that the principal metabolite, leuco-malachite green (LMG), is the main chemical found in fish treated with malachite green. This metabolite has a longer retention time inside fish muscle tissues and was reported to be carcinogenic (Sudova *et al.*, 2007).\

CONCLUSIONS

The occurrence of haemolytic *E. coli* isolated from cultured African catfish in commercial fish farms in Kano Metropolis was 69.3 %. Most of the haemolytic *E. coli* isolated from cultured *Clarias gariepinus* were resistant to chloramphenicol (70 %), furaltadone (80 %), Gentamicin (70 %), Amoxycillin (50 %), Streptomycin (40 %) Erythromycin (50 %), Nitrofurantoin (80 %), Tetracycline (50 %), Penicillin (90 %), and Doxycycline (50 %). The occurrence of residues of chloramphenicol, furaltadone and their metabolites in the fillets of cultured African catfish in the study area was zero (0). Most fish farmers in the study area lack knowledge of antibiotic resistance and rational drug use. Further studies should be conducted to characterize the *E. coli* isolated from cultured *Clarias gariepinus* in the study area. Other banned drugs which were not screened in this study should also be surveyed in *Clarias gariepinus* in the study area. There is a need to educate fish farmers in Kano on the rational use of antimicrobial agents in fish.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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