ENROFLOXACIN RESIDUES IN EDIBLE TISSUES OF FISH IMPORTED INTO NIGERIA

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Abstract
Drug resistance is a problem in the medical world today. It makes treatment difficult and void of expected results. Sub-lethal exposure to antibiotics is one of the major causes of drug resistance and the development of antibiotic-resistant strains of pathogenic organisms. This study explored the possibility of enrofloxacin residues in edible fish tissues from commonly imported and consumed fish species in Nigeria: Tilapia (Oreochromis niloticus), Horse mackerel (Trachurus murphyi), yellow croaker (Micropogonias furnieri), and Atlantic Mackerel (Scromber scombrus). Fish samples acquired from markets in Lagos were subjected to residue tests using the Green Spring® (Shenzhen Lvshiyuan Biotechnology Co. Ltd) Enzyme-Linked Immunosorbent Assay (ELISA) kit for Enrofloxacin. Randomly selected countries of import were; Argentina, Chile, China, Faroe Island, Iceland, Peru, and Uruguay. Results showed all studied samples having residues of enrofloxacin above internationally acceptable Maximum Residue Limits (MRLs). Croaker from Argentina recorded a significantly high residue level (0.650 ppm); Horse mackerel from Peru had the lowest residue level (0.12ppm). Indicating sub-lethal indirect exposure of consumers to enrofloxacin (none licenced aquaculture drug). Stringent attention is hereby recommended in investigating fish imports for antibiotic residues irrespective of the source to protect consumers' health.
Keywords: Antimicrobials, Pollution, food.

INTRODUCTION

Antibiotics are essential for preventive and therapeutic medicine, the use of which is expected to be with professional guidance to prevent abuse and misuse. In animal production and aquaculture, antibiotics are not only used for treatment and prevention of diseases, they are sometimes employed as growth promoters (Omotoso and Omojola, 2015). Drugs administered to animals directly or indirectly especially close to the harvest or slaughter time end as residues in the animal tissues thereby constituting health risk to consumers (Dipeolu, 2002).

Issues of drug resistance have raised serious concern in the public health industry over the years with indiscriminate use of drugs and self-medication considered as the primary cause of resistance. Studies have shown that residues of antibiotics in food consumed by humans are potential sources of sub-lethal doses that also contribute to drug resistance (Adeyemo, 2015).

The quinolones are very effective broad-spectrum synthetic antibiotics employed both in human and veterinary practice for the treatment of various systemic infections (Dordevic *et al.*, 2009), especially as last resort. This group which includes enrofloxacin, ciprofloxacin, and ofloxacin, has been of restricted use in food animals including aquaculture in recent years due to the effect of possible residues in food animals transferable to human consumers and their suspected carcinogenic tendencies (USFDA, 2006; CFIA,2015).

Studies on enrofloxacin and ciprofloxacin residues in fish muscles have been more related to the direct introduction of the drugs either on experimental conditions or its use in aquaculture production (Weihai Xu *et al.*, 2006; Hanwen *et al*., 2010; Mensah *et al*.,2019). Reports have pointed environmental pollution such as waste runoffs from animal and fish farms, treatments on marine aquaculture, human and general pharmaceutical wastes, municipal sewage treatment plants as a means by which residues of drugs and other toxic substances are introduced to aquatic life (Adeyemo *et al.*, 2010).

International trade and globalization of the food market have allowed for fish to be interchanged between countries. Nigeria imports fish in frozen form from several countries to meet up with her consumption demands (Fishsite,2015), and these products are presented for sale in open markets in the country, hitherto not much studies have been reported on the antibiotic residue status of most of these imported fish species. This study was designed to investigate the presence and quantify the level of enrofloxacin residues in fish muscles said to be harvested from the wild and imported into Nigeria intended for human consumption.

METHODOLOGY

Sampling

Selected fish species imported for food into Nigeria such as Tilapia (*Oreochromis niloticus*) from China, Horse mackerel (*Trachurus murphyi*) from Chile and Peru, Yellow croaker (*Micropogonias furnieri*) Argentina and Uruguay, and Atlantic Mackerel (*Scromber scombrus*) from Iceland and Faroe Island were purposively sampled from two major fish markets in Lagos state Nigeria;

Ijora fish market (6.470o N, 3.378oE) and Mile 12 market (6.607oN, 3.398oE) based on availability in original packaging. A total of 126 fish sampled; six batches of each species were sampled in triplicates from each country of origin irrespective of the species.

Extraction and analysis procedures

Green Spring® (Shenzhen Lvshiyuan Biotechnology Co. Ltd) ELISA kits for Enrofloxacin was used complying strictly with manufacturer's instructions for extraction.

Extraction was of a 20g homogenate from each sampled fish, 2 ± 0.05 g sample was taken into 50 mL centrifugal tube, 8 mL of N-hexane/ dichloromethane solution, prepared at ratio 1:3 was added and shaken in upside and down pattern vigorously for 5 min, centrifuged at above 4000 rpm at 15 ℃ for 10 minutes, then 2 ml of the supernatant was aspirated into a clean dry container and blown to dry with nitrogen at 50℃.

The dry residues were dissolved in 1 ml of the diluted redissolving solution into a test tube, and 1 ml of N-hexane was added and mix for 30s, before the mixture was centrifuged at above 4000 r/min at 15℃ for 5min, after which 50 µL of the lower layer was taken for further analysis.

With all the reagents at room temperature (20-25 ℃) and using Pre-numbered micro-well plates according to samples and standard solutions; the procedure was performed in duplicate as follows: 50 µl of the sample or standard solution were added to separate duplicate wells, then 50 µl of the enzyme conjugate and 50 µl of the antibody working solution was added to each well. This was mixed by shaking gently, and the microplate was sealed with the cover membrane and incubated at 25℃ for 1 hour.

After which liquid content was poured out of the wells, and the microplate washed with the washing buffer at 250µL/well four times by soaking the wells with the washing buffer for 15-30 seconds. The plate was then air-dried.

For Coloration: 50 µL of substrate A solution and 50 µL of B solution were added to each well. Mixed properly by shaking gently, and incubated at 25℃ for 15 min in the dark.

Then 50 µL of stop solution was added into each well and mixed by shaking gently before the OD values were read using the microplate reader. The OD values were read at the dual-wavelength of 450/630 nm within 5 minutes after the addition of the stop solution. The resulting value was then multiplied by the dilution fold (x2). This procedure was repeated for all samples collected and the results obtained were used to plot the standard curve for enrofloxacin in parts per billion(ppb).

RESULTS

The linear standard curve for Enrofloxacin standard solution using the ELISA kit is presented in figure 1 below**.**

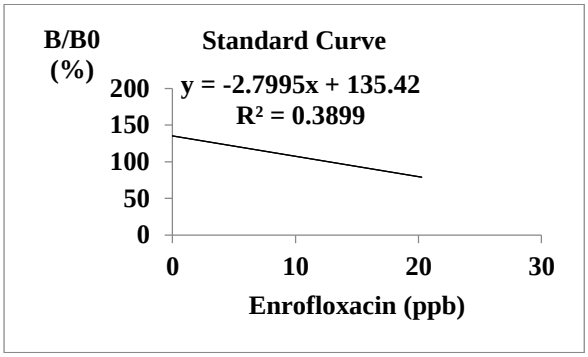

Figure 2 showing the mean Enrofloxacin values in fish samples from all eight countries of origin. All species of fish sampled and all fishing zone origins had residues of enrofloxacin higher than the internationally acceptable maximum residue limit of 0.1ppm allowed in the European Union as seen in Table 1(Addendum).

M. furnieri from Uruguay had the lowest residue level, while M. *furnieri* from Argentina had the highest enrofloxacin residue level.

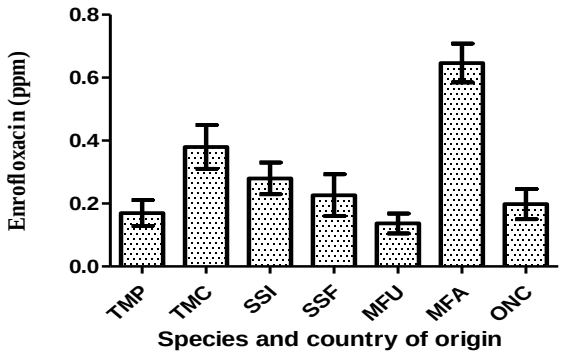

Figure 2: Mean Enrofloxacin residue levels in fish samples

TMP- *T. murphyi* Peru, **TMC**= *T. murphyi* Chile,
SSI= *S. scombrus* Iceland, **SSF**= *S. scombrus* Faroe Is.,
MFU= *M. furnieri* Uruguay,
MFA= *M. furnieri* Argentina,
ONC= *O. niloticus* China

DISCUSSION

The presence of antimicrobial residues in food products has been an issue of public health concern, not only due to the emergence of antibiotic-resistant strains of pathogenic bacteria organisms, other deleterious effects include the transfer of antibiotic-resistant bacteria to humans, immunopathological effects, carcinogenicity (sulphamethazine, oxytetracycline, and furazolidone), mutagenicity, nephropathy

(gentamicin), hepatotoxicity, and reproductive disorders. Chloramphenicol has been incriminated in bone marrow toxicity, and penicillin with allergy (Adeyemo *et al.,* 2011). The WHO in 2011 referred to this development as one of the most serious risks to human health.

According to the finding of Dairo *et al.*, (2004), enrofloxacin gradually depletes from fish tissue over time once the exposure is withdrawn, but this is on the condition that the fish is alive in an environment that does not pose further exposure. Results from this study corroborate the fact that there is no residue depletion after death and during storage otherwise *S. Scombrus* from FAO zones 27,13,209 that was in storage for up to 12 months before they were sampled would have had the lowest residues level (see addendum table 2). All samples had travelled several months (minimum of two months) under cold storage conditions before their sampling date.

Fish samples used in this study except for the *O. niloticus* from China that is declared as farmed were all from the wild as claimed in the packaging of the products. Therefore, the presence of residues of enrofloxacin may not be attributed to the direct application for therapeutic purposes; this can be an indication of pollution of the fishing zones by runoffs of waste from nearby communities. *M. furnieri* from Argentina that had the highest enrofloxacin residue was declared to be from the same South-western Atlantic (FAO zone 41) with *M. furnieri* from Uruguay that had the lowest enrofloxacin residue level, this brings to mind possible trawling beyond the boundary lines tending more towards the local community and or Marine culture use of this antibiotics. Detection of enrofloxacin residues in all imported fish samples already presented in the market for consumers in Nigeria corroborates the findings of other researchers in other countries where residue monitoring is absent or inadequate.

CONCLUSION

In the absence of law enforcement, crimes thrive, if the standards of the United States, Canada, and other developed countries were to be considered, the level of enrofloxacin residues in these fish products intended for human consumption would have earned them a total rejection and probably a sanction from those locations. Implying that the quality of the studied species imported from these water zones was below an acceptable standard, hence the health of consumers is being compromised with regular consumption of these products. Therefore, it is recommended that more stringent monitoring of fish imported and intended for human consumption irrespective of the source be carried out by the appropriate authorities as a measure of safeguarding the health of consumers. Regular data collection over time will also guide adequate legislative cover for the relevant enforcement agencies.

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Table1: Comparing enrofloxacin residue levels obtained (ppm) with international MRLs

Country	Species of fish	Enrofloxacin	References
Argentina	*M. Furnieri*	0.65	This study
Uruguay	*M. Furnieri*	0.14	"
Faroe Island	*S. Scombrus*	0.23	"
Iceland	*S. Scombrus*	0.28	"
Chile	*T. murphyi*	0.38	"
Peru	*T. murphyi*	0.12	"
China	*O. niloticus*	0.20	"
Canada	All	ND	CFIA (2015)
Korea	All	0.1ppm	KFDA Food code, 2011
EU	All	0.1ppm	EEC (1990)

Table 2: Sample codes, Countries and FAO fishing zones

Country	Fish type	Code	FAO zone	Min. storage time (months)	Max. storage time
Peru	Horse Mackerel	TMP	87 Southeast Pacific	4 months	10 months
Chile	Horse Mackerel	TMC	87 Southeast Pacific	3 months	10 months
Iceland	Mackerel	SSI	27 Northeast Atlantic	2 months	10 months
Faroe Island	Mackerel	SSF	27,13,209	4 months	12 months
Uruguay	Croaker	MFU	41 South west Atlantic	6 months	8 months
Argentina	Croaker	MFA	41 South west Atlantic	3 months	9 months
China	Tilapia	ONC	Farmed	2 months	7 months