Occurrence of antibiotic residues in raw fish Clarias gariepinus and Oreochromis niloticus from intensive rearing system in Benin

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Occurrence of antibiotic residues in raw fish *Clarias gariepinus* and *Oreochromis niloticus* from intensive rearing system in Benin

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Abstract

An observational cross-sectional study was carriedy out to investigate antibiotic residues in *Clarias gariepinus* and *Oreochromis niloticus* from the intensive rearing system in Southern Benin. The targeted antibiotic families were tetracyclins, amphenicols, beta-lactams and macrolides. One hundred and forty-four (144) samples were used per antibiotic family for residue detection in the fish muscle, making 576 treated samples. The Charm II method based on radioimmunoassay was used. The results showed an overall residue prevalence of 11.1%, which is attributable to tetracyclines alone. There was no residue of the other antibiotic families in the treated fish muscles. The adult fish were the only contaminated age group with 22.2% residue prevalence compared to the fingerlings ($p < 0.05$). Likewise, the antibiotic residues were significantly ($p< 0.05$) more prevalent in *Clarias gariepinus* (16.7%) than in *Oreochromis niloticus* (5.6%). The adult fishes are generally fattier than the young ones, and that can lower the fish body antibiotic elimination capability. This first detection of tetracyclines residues in fish produced in Benin shows the need to rule and control antibiotic use in the developing fish industry to preserve consumers' health.

Keywords: Tilapia, African catfish, fish farm, antibiotic residue, Benin

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Introduction

Aquaculture is a worldly fast-growing animal production sector. Worldwide, fish provide 15% of animal production source protein to more than three billion people and play a vital economical role by contributing specifically to unemployment rate reduction (FAO, 2011). In Benin, food safety is a big concern because of the high population increase rate of 3.5% annually (INSAE, 2013). The efficient way of addressing this problem is the intensification of animal production including the fish production.

Fish production in Benin is at its earliy intensification stage. The use of antibiotics as food supplements for disease prevention and treatment and as growth promoters (Reda *et al.*, 2013; Pham *et al.,* 2015) is a common practice. However, such use of antibiotics without veterinary control lead inevitably to the presence of antibiotic residues in the animal-derived products and by-products (Mensah *et al.,* 2014). The presence of antibiotic residue in fish products was well documented. The utilization of antibiotic products in aquaculture is prejudicial to the aquatic environment and aqualife on one hand, and on the other hand, to the fish products consumers due to the toxicity risk of antibiotic residues (Cabello, 2006; Olatoye and Basiru, 2013; Dhaouadi *et al.,* 2015). Unfortunately, no scientific work has been done to date on antibiotic residues in intensive fish farming products in Benin.

The current study was carried out in an observational cross-sectional design to assess the occurrence of antibiotic product residue in fish from the intensive rearing system in Southern Benin.

Material and methods

Fish sample collection

Fish were collected in an intensive fish production farm in a southern region of Benin. The two fish species produced in this farm were *Clarias gariepinus* (African catfish) and *Orechromis niloticus* (Tilapia of the Nile River). The farm includes two major farm units located in two different districts. The first unit was the hatchery and catfish fattening site located in Ouèdo, and the second was the tilapia growing farm located in Pahou. The fish were fed with the commercial feed.

One hundred and forty-four (144) fishes were randomly collected: seventy-two (72) per species and thirty-six (36) for each age group, adult and growers.

The collected fish samples were transported in the ice boxes to the Central Laboratory of Sanitary and Safety Food Control of the Ministry of Agriculture, Livestock and Fishery of Benin, and then stored at -20°C until the laboratory analysis.

Laboratory analysis

Four antibiotic families were targeted in the laboratory analysis (Table 1). These antibiotics were broad spectrum antibiotics likely to be used by the fish farmers. Consequently, four samples were taken from each fish to search for amphenicols, beta-lactams, macrolides and tetracyclines antibiotic residues with a total of 576 fish samples made available for the test.

Charm II screening test was used for antibiotic residues detection in the fish samples. This is a radioimmunoassay with the competition for the specific binding sites between drug residues and radioactive tracers. The amount of tracer that binds to the receptor sites is measured and compared to the previously determined control point (Table 1). Control point is calculated from the average value obtained running 6 tests of negative controls which values must not deviate more than 15% from the average. The control point is obtained by subtracting 40% from the negative control a verage (Charm Sciences Inc., 2019).

Table 1: Detection kit capacity for antibiotic residue (Charm Sciences Inc., 2019)

Antibiotic familiy	Antibiotics	Cut-off values (ppb)
Amphenicols	Chloramphenicol	10
	Florfenicol	-
Beta-lactams	Amoxillin	50
	Ampicillin	40
	Cefalexin	200
	Cefquinone	200
	Ceftiofur and metabo	400
	Cephapirin	40
	Cloxacillin	500
	Dicloxacillin	300
	Oxacillin	500
	Penicillin G	50
Macrolides	Erythromycin	100
	Lincomycin	400
	Pirlimycin	600
	Spiramycin	400
	Tilmicosin	50
	Tulathromycin	100
	Tylosin	400
Tetracyclines	Chlortetracycline	100
	Oxytetracycline	100
	Tetracycline	25

Statistical analysis

The Pearson Chi square test (without Yates continuity correction) was used to compare the occurrence of antibiotic residues in the fish muscles between fish age groups (adults and fingerlings) and species (*C. gariepinus* and *O. niloticus*) in R 3.5.1.

Results

Only tetracycline residues were found in 11.1% of the fish muscle samples regardless of species and age group. All the fish muscle samples were free from amphenicols, macrolides and beta-lactams' residues.

Considering age groups, only the muscle samples of adult fish (22.2%) contained residues of this antibiotic family. No residue was found in fingerlings muscle samples. Otherwise, such tetracycline residues were three times more prevalent ($p<0.05$) in *Clarias gariepinus* (16.7%) than in *Oreochromis niloticus* (5.6%).

Discussion and conclusions

In this study, a radioimmunoassay is used for residue detection because it is a fast and reliable method compared with microbiological, immunological or physicochemical methods for antibiotic residue screening and quantification in the animal muscles.

Several studies reported the presence of antimicrobial residues in food of animal origin in Benin (Mensah et *al.,* 2014). However, no investigation or quantitative analysis has been done on the presence of antibiotic residues in fish produced and consumed in this country. In Ibadan, Nigeria, antibiotic residues have been detected using Premi®Test kit in 52.5% of ready-to-eat *C. gariepenius* muscle collected from restaurants and farms (Olatoye and Basiru, 2013). The presence of antibiotic residue in the fish muscle samples of the current study is in line with the results of the above mentioned study, and can be explained by the routine use of antibiotics in fish diets in the intensive production system. This might result from adding antibiotics to the locally-produced foods just before harvesting in order to boost growth (Reda *et al.*, 2013) and prevent disease and stress during live-fish transportation (Romero *et al.,* 2007; Olatoye and Basiru, 2013; Pham *et al.,* 2015).

Tetracyclines residues were the only antibiotic residues found in this study. Similarly, tetracyclines residues had been detected in, respectively, 7.84% and 30% of locally-produced fish in Vietnam (Pham *et al.,* 2015) and Nigeria (Olatoye and Basiru, 2013). Tetracyclines seem to be one of the most commonly used antibiotic families in fish production (Olatoye and Basiru, 2013; Reda *et al.*, 2013).

Moreover, in this study, only adult fish muscles tested positive to antibiotic residues , irrespectively of the species. The body adipose tissue is known to be susceptible to the drug residue in general, so that it can significantly lower the antibiotic pharmacokinetic activities of the body

organs. This can explain the difference between the two age groups, adult and fingerlings, in antibiotic residue prevalence. Two fish species investigated in this study are positive to tetracyclines residues, with a higher prevalence in *C. gariepinus* than *O. niloticus*. This may be attributable to different disease management since drug administration, and/or blood composition of *C. gariepinus* and *O. niloticus* (Hamid *et al.*, 2013), which can influence tetracyclines pharmacokinetic. Likewise, tetracyclines residues have been detected in the samples from those two fish species in the respective concentrations of 1.98 and 3.08 ppm in Ibadan, Nigeria (Olusola *et al.*, 2012). Eventhough the fish samples collected in our study are free from amphenicols, macrolides and beta-lactams residues, chloramphenicol (an amphenicol) residues have been found in the *C. gariepinus* muscle sample in Nigeria (Olusola *et al.*, 2012). The presence of residues of other antibiotic families like quinolones in the *O. niloticus* muscle sample has also been mentioned (Xu *et al.*, 2006; Pham *et al.*, 2015). Withdrawal time of 3 to 42 days depending on the antibiotic used and water temperature, is needed to avoid the presence of antibiotic residues above maximum residue limit (MRL) in fish (Chafer-Pericas *et al.,* 2010).

This first detection of tetracyclines residues in raw *C. gariepinus* and *O. niloticus* produced in Benin proves that antibiotics are misused in fish farming as in other animal farming (Mensah et al., 2011). Fish consumers in this country are then exposed to many risks related to the presence of antibiotic residues in food like antibiotic resistance, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity or allergy (Mensah *et al.*, 2014). Local fish industry should be included in a national system for control of drug residues in food of animal origin to preserve consumers' health.

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Prisustvo antibiotskih rezidua u sirovoj ribi *Clarias gariepinus* i *Oreochromis niloticus* iz intenzivnog uzgoja u Beninu

SAŽETAK

Izvedeno je opservacijsko presječno istraživanje kako bi se utvrdilo prisustvo antibiotskih rezidua u ribama *Clarias gariepinus* i *Oreochromis niloticus* iz intenzivnog uzgoja u južnom Beninu. Ispitivani su antibiotici iz skupine tetraciklina, amfenikola, beta-laktama i makrolida. U istraživanju su ispitivana sto četrdeset i četiri (144) uzorka po antibiotskoj skupini, ukupno 576 uzoraka na prisustvo rezidua u ribljim mišićima. Od metoda je korišten Charm II radioimuno test. Rezultati su pokazali prisustvo rezidua tetraciklina od 11,1%. U ispitivanim uzorcima ribe nisu otkrivene rezidue ostalih antibiotskih skupina. Jedina kontaminirana skupina riba su bile odrasle jedinke sa prevalencom od 22,2% u odnosu na mlade jedinke ($p < 0.05$). Prevalenca antibiotskih rezidua je statistčki signifikantnija ($p< 0.05$) kod vrste *Clarias gariepinus* (16.7%) u odnosu na *Oreochromis niloticus* (5.6%). Odrasle jedinke riba generalno imaju više masnog tkiva nego mlade jedinke, što može sniziti eliminacijsku sposobnost tijela. Prvo otkrivanje prisustva antibiotskih rezidua u ribama uzgojenim u Beninu je ukazalo na potrebu kontrole upotrebe antibiotika u ribogojstvu u svrhu zaštite zdravlja potrošača.

Ključne riječi: Tilapija, Afrički som, ribnjak, antibiotski reziduum, Benin