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Antibiotic Residues in Commercially Available Freshwater and Marine Fish: A Risk Assessment

Maysa Mohammed El Asely[1*], Gehad Fathy Fath Elbab[2], Fahim Aziz ELdein Shaltout[1*]

[1]Food Control Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt
[2]Food Hygiene Department, Animal Health Research Institute (AHRI), Egypt

***Correspondent Author:** Fahim.shaltout@fvtm.bu.edu.eg; mohammedmaysa69@gmail.com

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ABSTRACT

A total of 150 fish samples were obtained from two sources: the local fish markets, for specifically the Nile tilapia (*Oreochromis niloticus*) specimens in addition to the African catfish (*Clarias gariepinus*), with 25 samples collected for each species. The second source is the farms raising fish for export, including meager (*Argyromus regiym*), the European sea bass (*Dicentrarchus labrax*), the European eel (*Anguilla anguilla*), and flathead grey mullet (*Mugil cephalus*), with 25 samples for each species. The data collection process involved the use of the fundamental random sampling methodology. The sampling was conducted between November 2022 and July 2023 addressing various aquaculture sites in Kafr Elshiekh Governorate, Egypt. The samples were immediately transferred to the laboratory for antimicrobial screening using an icebox, without any delay. Antibiotic residues belonging to the B lactam group were identified in the Nile tilapia at a prevalence rate of 4.67% among the total sample size of one hundred and fifty fish specimens. No traces of other antibiotic residues, including tetracycline, aminoglycosides, fluoroquinolones, and macrolides, were found in the samples of the Nile tilapia. The presence of all chosen categories of antibiotics was not seen in any of the surveyed samples, which included African catfish, meager, flathead grey mullet, European eel, and European sea bass. In conclusion, the current findings indicated that 4.67% (seven samples) of the surveyed samples contained detectable antibiotic residues, while 95.33% (143 samples) did not show any trace of antibiotic residues.

INTRODUCTION

Fish individual is a widely consumed dietary item for a significant worldwide population. Fish are rich in high-quality protein, and lipids, as well as several essential vitamins and minerals. In addition, fish oil is abundant in omega-3 fatty acids, which have positive effects on heart disease and dermatitis **(Edris *et al*., 2017; Pandey & Saad *et al*., 2022; Upadhyay, 2022; Farage *et al*., 2023b)**. It was assumed that fish and their products account for around 17% of the animal protein consumed by humans and approximately 7% of the total protein consumed overall. The rate of fish consumption on a global scale has experienced a notable acceleration and significant increase due to the rise in earnings and the growing recognition of the health advantages linked to eating fish **(Hassan & Shaltout, 2004; Anderson *et al*., 2017)**. To sustain this upward trend, there has been a significant expansion in the field of aquaculture. Moreover, the aquaculture industry has emerged as the most rapidly expanding sector in food production, contributing to socioeconomic development, poverty alleviation, and the creation of numerous employment possibilities **(Hassan *et al.*, 2014; Salayo *et al*., 2022; Farage *et al*., 2023a; Soleimani & Khanjani, 2023)**.

In Egypt, fish production comes from natural fisheries and fish farms. Natural fisheries provide approximately 455 thousand tons, while fish farms account for approximately 1443.2 thousand tons **(Anees *et al*., 2023; Khalil, *et al*., 2023)**. Moreover, it is worth noting that the aquaculture industry plays a significant role of the overall fish output, hence ensuring the provision of approximately 580,000 employment opportunities for individuals working in the designated sector (**Shaalan *et al*., 2018)**.

The development of intensive aquaculture production systems was undertaken with the objective of achieving high fish production yields for meeting the growing demand for fish **(Cremer *et al*., 2014)**. Nevertheless, it is important to note that fish cultured in intensive and semi-intensive systems may experience significant susceptibility to diseases due to the presence of overcrowding and various stressors **(Firmino *et al*., 2019; Ciji & Akhtar, 2021)**. In the field of fish farming, infectious diseases pose a significant problem that is commonly linked to elevated rates of morbidity and mortality **(Lulijwa *et al*., 2020)**. The predominant infectious agents affecting aquatic animals in the context of aquaculture production are bacterial pathogens, accounting for 54.9% of cases. Viruses rank second, contributing to 22.6% of infections, while parasites and fungi account for 19.4% **(Surachetpong *et al*., 2020)**. Hence, antibiotics such as tetracyclines, sulfonamides, trimethoprim (TMP), quinolones, β-lactams, phenicols, and fluoroquinolones have been widely used in the field of aquaculture to treat and prevent infections **(Manage, 2018; Gorito *et al*., 2022)**. In addition, the inclusion of small amounts of growth promoters was employed to enhance feed efficiency and stimulate weight gain **(Magouras *et al*., 2017; Cuong *et al*., 2018)**.

The excessive and improper utilization of antibiotics in aquaculture has the potential to give rise to health issues in both humans and animals **(Manage, 2018)**. These concerns include the emergence of antibiotic resistance, as demonstrated by **Liu *et al*. (2013)**, as well as the occurrence of allergic reactions in individuals with hypersensitivity, such as penicillin allergies, and even the possibility of anaphylactic shock. Furthermore, the administration of certain antibiotics, such as sulfamethazine, oxytetracycline, furazolidone, gentamicin, and chloramphenicol has been associated with the induction of more severe pathologies, including cancers, nephropathy, bone marrow toxicity, teratogenicity, and mutagenic effects **(Baynes *et al*., 2016; Bacanlı & Başaran, 2019; El-Ghareeb *et al.*, 2019)**. The objective of this study was to detect the presence of antibiotic residues in various fish species in Egypt, and subsequently, the implications for public health regarding the occurrence of antimicrobial residues in fish were further discussed.

MATERIALS AND METHODS

1. Collection of samples

From the local market, a total of 150 fish samples were collected, including the Nile tilapia (*Oreochromis niloticus*) and the African catfish (*Clarias gariepinus*) with 25 samples for each. Additionally, fish from farms intended for export, such as meager *(Argyromus regiym)*, European sea bass *(Dicentrarchus labrax)*, European eel (*Anguilla anguilla*), and flathead grey mullet *(Mugil cephalus)*, were also sampled, with twenty-five samples from each species. Samples were gathered using the basic random sampling approach. The sample size was obtained using a confidence level of 90% and a margin of error of 10%. The study involved the collection of samples from farmed fish that were sold in fish markets and from the fish farms with history of using antibiotic (self-communications). The cultured tilapia exhibited an average body weight of 200± 20g, whereas the cultured catfish had an average body weight of 440± 75g. The collection of samples took place from November 2022 to July 2023 from different aquacultures in Kafr-Elshiekh Governorate, Egypt. The samples were promptly transported to the laboratory for antimicrobial screening using a chilled icebox, without any delay.

2. Screening for antimicrobial residues using a microbial inhibition assay

2.1. Solutions used in bioassay

A. 0.1M phosphate buffer, pH 4.5+ 0.1
B. 0.1M phosphate buffer, pH 6.0+ 0.1
C. 0.1M phosphate buffer, pH 8.0+ 0.1
D. 0.2M phosphate buffer, pH 8.0+ 0.1
E. Butterfield's phosphate buffer, pH 7.2
F. Reference antibiotics as needed - Minimum: Tetracycline hydrochloride, penicillin G potassium salt, streptomycin sulfate, erythromycin, neomycin sulfate

2.2. Media used in bioassay (HIMEDIA laboratories, USA)

A. Antibiotic medium 4 (or antibiotic medium 2 with dextrose)
B. Antibiotic medium 5
C. Antibiotic medium 8
D. Antibiotic medium 11
E. Plate count agar

2.3. Test organisms

Microorganisms used for the bioassay system

A) *Kocuria rhizophila* (*K. rhizophila*) formerly (*Micrococcus luteus*), ATCC 9341a (KR)
B) *Kocuria rhizophila* (*K. rhizophila*), ATCC 15957 (KRER)
C) *Staphylococcus epidermidis* (*S. epidermidis*), ATCC 12228 (SE)
D) *Bacillus cereus* var. *mycoides* spores (*B. cereus*), ATCC 11778 (BC)
E) *Bacillus subtilis* spores (*B. subtilis*), ATCC 6633 (BS)

2.4. Performing the seven plate bioassay

The following plates were used with the extracts listed to test for each of the standard antibiotic residues. The placement of the plates were listed in this sequence for the full assessment.

Plate 1 BC (tetracycline detection – pH 4.5 buffer extract)
Plate 2 KR (beta-lactam detection – pH 6.0 buffer extract)
Plate 3 KR+P (penicillin confirmation – pH 6.0 buffer extract)
Plate 4 BS (streptomycin detection – pH 8.0, 0.1M buffer extract)
Plate 5 KR-11 (macrolide detection – pH 8.0, 0.2M buffer extract)
Plate 6 KRER (erythromycin confirmation – pH 8.0, 0.2 M buffer extract)
Plate 7 SE (aminoglycoside detection – pH 8.0, 0.1M buffer extract)

2.5. Method of screening

A microbial inhibition assay was performed for the detection, identification, and quantitation of antibiotic residues according to the technique described by the United States Department of Agriculture “USDA” and Food Safety and Inspection Services **(FSIS, 2011)**. The microbiological system consists of the detection, identification, and quantitation of several antibiotic groups, such as beta-lactams, tetracycline, aminoglycosides, flouro-quinolones, and macrolides following the steps briefly; tissues were handled so that freezing and thawing are kept to a minimum. The muscle tissue samples were excised and placed in bags which were labeled for sample identification. 10g of the tissues were diced into 0.5cm pieces, followed by dispensing 40± 1.0ml of the appropriate buffer into each bag and homogenized using a blender for 60 seconds. After stomaching or mixing, the tissue was allowed to settle for a minimum of 45 minutes before use. The extracts were stored in refrigeration for 24h and then frozen for an additional 14 days for further testing. Seven plates were filled with 200± 4µl of the appropriate buffered sample/well. This process was repeated for the additional samples. The following antibiotic reference standard (SRs) was used in one well each day while the screen test was run: Plate 1, tetracycline; Plates 2 and 3, penicillin; Plate 4, streptomycin; Plates 5 and 6, erythromycin; Plate 7, neomycin or gentamicin. 200± 4µl of the SR concentration was pipetted into the test well. Upon screening the testing samples, Sensi-Discs may be used in place of using reference dilutions on each plate. Plates 1 through 6 was incubated at 29± 1^{o}C for 16 to 18h. While, plate 7 was incubated at 37±

1^{o}C for 16- 18h. After incubation, the zones of inhibition (or absence of zones) on each of the seven plates were recorded.

2.6. Calculating the concentration of antibiotic residue in tissue

The zone sizes on the test plate were compared to the standard curve to calculate the residue concentration in the test sample.

3. Statistical analysis

The statistical analysis and computations were performed using the Excel application. The findings were presented in terms of the mean, standard error, minimum, and maximum. The statistical software package SPSS was used to assess Pearson's correlation coefficient between the concentrations of the antibiotics that had been found. The level of significance was determined at P= 0.05 and P= 0.01.

RESULTS

The data in Table (1) reveal that the mean concentration of B lactam group was 3.2ppb ±1.01 in the Nile tilapia samples from local fish markets in Kafr El Shiekh Governorate, with maximum and minimum concentrations of 14 and 8ppb. While, other antibiotic groups of bioassays (Tetracycline, macrolide, aminoglycoside, and floroquinolone) were not detected.

None of the five antibiotic group residues have been identified in the bioassay of African catfish samples obtained from local fish markets in Kafr El Shiekh Governorate. However, it was found that the screening of the five antibiotic groups in meagre, European sea bass, European eel, and flathead grey mullet did not identify any residues of the bioassay in fish farms for export in Kafr El Shiekh Governorate (Table 2).

The standard curve obtained from the standard penicillin (B lactam group of antibiotics) is shown in Fig. (1) with the linear equation of y= 0.16x + 7.7. Here, y= Inhibition zone (mm) and x= Concentration of the penicillin (ppb) and the correlation coefficient (R^2) = 0.9846 showing the linearity. Based on the data presented in Table (3) and Fig. (2), the results of the microbiological inhibition assay indicate that antibiotic residues were identified in 28% (seven samples) of the Nile tilapia samples investigated, whereas 72% (eighteen samples) showed no detectable levels of antibiotics. In contrast, the percentage of undetected antibiotic residues in the remaining samples collected, which included African catfish, meagre, European sea bass, European eel, and flathead grey mullet, was found to be 100% for each individual species.

In conclusion, the findings from Table (4) and Fig. (3) indicate that 4.67% (seven samples) of the surveyed samples contained detectable antibiotic residues, while 95.33% (143 samples) did not show any traces of antibiotic residues.

Table 1. Antibiotic residues in survey fish samples collected from local fish farms located in the Kafr El Shiekh Governorate using microbiological bioassay: (n= 50) (25 from each species)

Antibiotic group		The Nile tilapia	The African catfish
Tetracycline		ND	ND
B lactam	Min	8	
	Max	14	
	Mean	3.2	ND
	± SE	1.01	
Macrolide		ND	ND
Floroquinolone		ND	ND
Aminoglycoside		ND	ND

ND: Not Detected. SE: Standard error.

Min: Minimum. Max: Maximum.

Table 2. Antibiotic residues in survey fish samples collected from fish farms for export in Kafr El Shiekh Governorate: (n= 100) (25 for each species)

Antibiotic group	Meager	European sea bass	European eel	Flathead grey mullet
Tetracycline	ND	ND	ND	ND
B lactam	ND	ND	ND	ND
Macrolide	ND	ND	ND	ND
Floroquinolone	ND	ND	ND	ND
Aminoglycoside	ND	ND	ND	ND

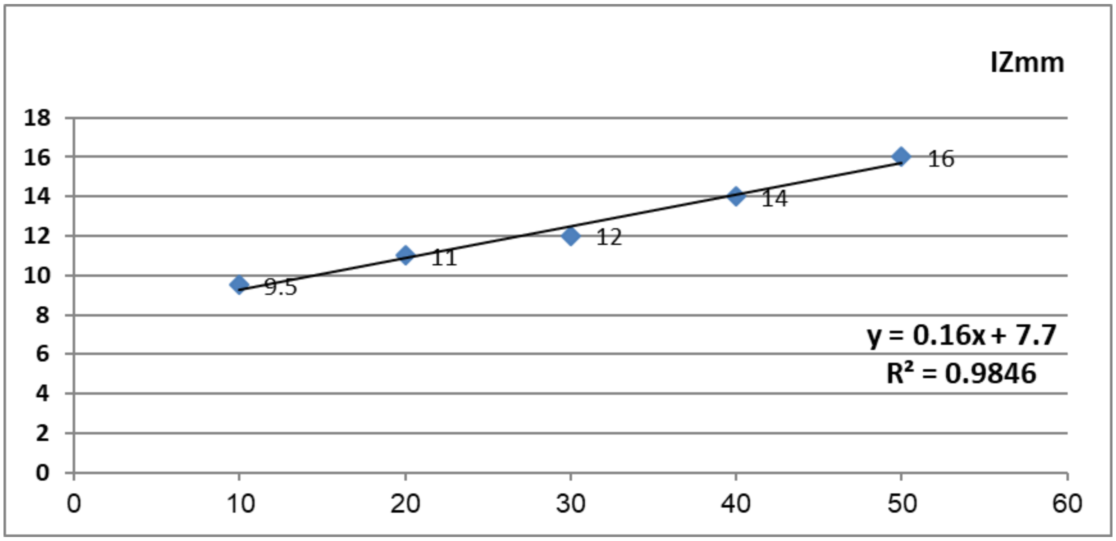

Fig. 1. Standard curve of B-Lactams

Table 3. Antibiotic residues of different species of fish in survey samples

Sample	Nile tilapia		African catfish		Meager		European sea bass		European eel		Flathead grey mullet	
	n	%	n	%	n	%	n	%	n	%	n	%
D - AB	7	28	0	0	0	0	0	0	0	0	0	0
ND - AB	18	72	25	100	25	100	25	100	25	100	25	100
Total	25	100	25	100	25	100	25	100	25	100	25	100

N: Number. %: Percent.

D - AB: Detected antibiotic. ND - AB: Not detected antibiotic.

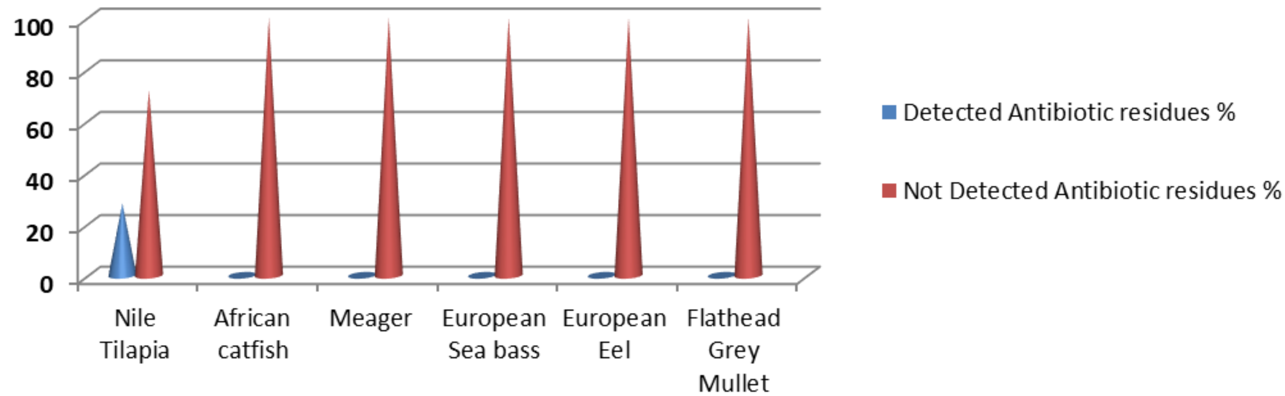

Fig. 2. Antibiotic residues of different species of fish in survey samples (n= 150)

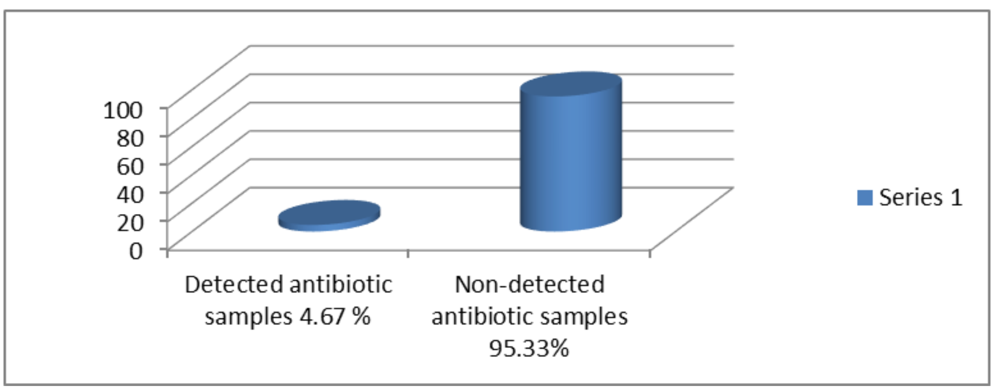

Fig. 3. Percentage of detected and not detected antibiotic residues of in survey fish samples (n= 150)

DISCUSSION

Antibiotics play a crucial role in the hygienic management of aquaculture facilities. The inappropriate use of antibiotics in aquaculture has the potential to result in the undesirable accumulation of pharmaceutical residues within the consumable flesh of fish, hence posing public health hazards to consumers. In contrast, fish are regarded as bioindicators for the presence of antibiotic contamination in aquatic settings. Antibiotics are employed within the field of aquaculture for a multitude of objectives, encompassing the management of bacterial ailments, augmentation of fish yield, and mitigation of financial detriments. Nevertheless, the potential risk to people arises from the misuse and overuse of antibiotics, mostly due to the escalating issue of antibiotic resistance and the detection of antibiotic residues in fish muscle tissues. To safeguard human health, it is imperative to undertake a series of measures, with the initial step involving the implementation of screening processes.

The current study aimed to assess the presence of antibiotic residues in a total of 150 fish samples through the utilization of a microbial inhibition assay. A total of 150 fish samples were collected from various fish farms, including both local and export. The sample population comprises six species of cultivated fish, namely the Nile tilapia, African catfish, meagre, European sea bass, European eel, and flathead grey mullet. Among these species, 4.67% of the samples were found to contain antibiotic residues. However, these residues did not surpass the maximum residue limit (MRL) of 50 parts per billion (ppb), as established by the National Food Safety Authority Resolution No. 13 (NFSA) in 2020, and the Codex Alimentarius Commission in 2021. In the present context, a total of twenty-five samples were used to represent the Nile tilapia. Antibiotic residues were identified in seven samples, accounting for about 28% of the samples analyzed. Among the remaining samples (143), a percentage of 33% exhibited no traces of antibiotic residues, whereas the remaining 67% did contain such residues. The study conducted by **Agoba *et al*. (2017)** in Ghana involved 63 fish farmers. Out of these participants, four individuals, accounting for 6.35% of the sample, reported the use of antibiotics. **Uchida *et al*. (2016)** detected a greater prevalence of antibiotic residues in fish and prawn samples obtained from marketplaces in Vietnam, with 55 out of 511 samples (10.76%) testing positive. **Yipel *et al*. (2017)** conducted monitoring of antibiotic presence in three distinct species of farmed fish (n= 75; rainbow trout, sea bass, gilthead sea bream) throughout five cities in Turkey. According to the findings of **Yipel *et al*. (2017)**, a significant proportion of the samples studied, specifically 33.3%, contained at least one residue of antibiotics. Furthermore, it was observed that 20% of the samples contained several residues of antibiotics.

While, **Mahmoudi *et al*. (2014)** found that, up to 56% of examined rainbow trout meat samples were contaminated with tetracycline (TC), chloramphenicol (CAP), and sulfonamide residues. Similar to the previous results are those detected by **Ferdous *et al*. (2019)** in Bangladesh who found that 56.9% of examined fish samples contained antimicrobial residues. **Pham *et al*. (2017)** monitored antibiotic use and residues in freshwater aquaculture in Vietnam and found that 68/94 (72.3%) of freshwater farms used at least one antibiotic at any time in the production cycle.

In Spain, **Hurtado de Mendoza *et al*. (2012)** determined sulfonamides and tetracycline residues in 16 out of 107 (14.95%) in the examined catfish samples. While in Egypt, **Morshdy *et al*. (2022)** found that 12/50 (24%) and 16/50 (32%) of the examined cultured tilapia and cultured catfish contained antibiotic residues. This result was higher than that detected in the current study 7/50 (14%). Differences in the percent of antimicrobial residues in the present study and other studies could be due to many reasons such as differences in fish species, source of fish, season of sampling, assayed method or farm practices, such as method of antibiotic administration, type and concentration of drug.

In a recent investigation, two distinct groups of fish were identified: fish intended for local market consumption, specifically the Nile tilapia and catfish. Out of a total of 50 samples analyzed, it was found that 7 samples or 14% of the total contained detectable levels of antibiotic residues. The second category comprises fish species (Meagre, European sea bass, European eel, and flathead grey mullet) intended for export, where no traces of antibiotic residue have been identified. This phenomenon can be attributed to the implementation of stringent laws and limitations on international trade, coupled with a lack of oversight over the domestic market.

CONCLUSION

In summary, the findings obtained from the present investigation have demonstrated the presence of antibiotic residues in the Nile tilapia, indicating contamination. To mitigate the presence of antibiotic residues, it is advisable to implement the following guidelines:

1. The consideration of the withdrawal period of antibiotics is critical to cease the administration of antibiotics before the marketing of fish.
2. The presence of veterinary supervision is essential on every farm to ensure the proper administration of antibiotics.
3. It is imperative that every fish farm has regular screening and rapid detection methods for antibiotic residues.

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