ANTIBACTERIAL RESIDUES IN CULTURED NILE TILAPIA (*Oreochromis niloticus*) IN THE LAKESHORE BARANGAYS OF LOS BAÑOS, LAGUNA, PHILIPPINES

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ABSTRACT

This study aimed to address a knowledge gap in the presence of antimicrobial residues in fish aquaculture products. Screening of food products from animal origin for the presence of antimicrobial residues has become a necessity due to the widespread use of these drugs as well as the development of resistant bacterial pathogens which has become a global issue. Tetracycline, ceftiofur, florfenicol, quinolone, streptomycin, and tylosin were screened in lateral muscle samples of Nile tilapia (*Oreochromis niloticus*) obtained in the coastal barangays of Los Baños, Laguna, Philippines. Using biochip immunoassay, the following results were obtained: tetracycline (100%), ceftiofur (100%), quinolone (83%), and florfenicol (9%). Streptomycin and tylosin were not detected in the samples. A minimum of two to a maximum of four classes of antibacterial were found in each sample. Although the residues did not exceed the established maximum residue limit (MRL), these still pose a health risk to consumers in view of widespread concern about antimicrobial resistance.

Keywords: antibacterial, drug residue, fish cage, Nile tilapia, Laguna de Bay

INTRODUCTION

The discovery of antibiotics has led to a dramatic reduction in the morbidity and mortality of infectious diseases. However, its extensive use and misuse have brought health risks to consumers. Residual antibiotics in animal products have caused hypersensitivity reactions, disruption of gut microbiota, and emergence of antibiotic resistant bacteria (Monteiro et al., 2018; Wistrand-Yuen et al., 2019). Antibiotic residues were detected in local chickens (Baldrias et al., 2008; Develos, 2015; Ang et al., 2017) and pork (Toralba et al., 2020) but not much is known about their occurrence in fish. Fish and fishery products in the Philippines have only been monitored for the banned antibiotics chloramphenicol and nitrofurans as well as cyanide and formaldehyde (BFAR, 2004).

Aquaculture remains a source of food and livelihood in municipalities surrounding Laguna de Bay, the largest lake in the country. There were 145 fish pen operators in the lake (Israel, 2007) that employed 5,112 people in 2006 (Israel, 2008). Concerns about the adverse impact of aquaculture on lake ecology have been raised (Israel, 2007). However, studies on the use of antibiotics in local aquaculture have been scarce despite apprehensions of its adverse implications on aquatic biodiversity and microbial communities, as well as on the emergence of drug-resistant bacterial pathogens (Lulijwa et al., 2019). This study was conducted to determine the presence of antibacterial residues in Nile tilapia (*Oreochromis niloticus*) from selected fish cages in Laguna de Bay along the municipality of Los Baños in the province of Laguna.

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MATERIALS AND METHODS

A total of 54 frozen adult Nile tilapia were purchased from fish cage operations in each of the following coastal barangays in Los Baños, Laguna: Bayog, Mayondon, Malinta, Baybayin, Bambang, and Tadlac (Figure 1) in November 2017. Approximately 10 g of the lateral muscle of each fish was placed in a labelled zipper bag and stored at -80°C. Each sample was thawed prior to processing, and 1 g was placed into a homogenization tube with two stainless steel balls. A working strength wash buffer (0.5 ml) was added to the sample which was then homogenized using the 1600 MiniG® for 15 seconds at 1300 rpm. Another 0.5 ml of working strength wash buffer was added to the homogenized sample, vortexed for 30 seconds, and centrifuged for 10 minutes at 4000 rpm. The supernatant (0.15 ml) was transferred to a microcentrifuge tube and diluted with 1.35 ml working strength wash buffer, for a final dilution of 1:20.

Assay diluent (100 µl) was pipetted to each biochip followed by the addition of 100 µL of sample. The biochips were placed in a thermostaker for 30 minutes for 370 rpm at 25°C to incubate. Conjugate (100 µl) was added to each biochip which was further incubated for 60 minutes for 370 rpm at 25°C. The biochips were then subjected to two quick wash cycles followed by four wash cycles with two-minute soaking time in between cycles. Around 350 µl wash buffer was then added to each biochip and left to soak for about 30 minutes. The wash buffer was discarded and the signal reagent (250 µl) was added to each biochip prior to covering the carrier with foil. The carrier was left to soak for 2 minutes. Thereafter, the samples were analyzed using Evidence Investigator™.

The biochip array used in this study can simultaneously screen for compounds under the following antibiotic families: tetracycline, ceftiofur, quinolone, florfenicol, streptomycin, and tylosin. The limits of detection in µg/kg were 4.8, 4.6, 5.0, 1.3, 14, and 0.9, respectively. The florfenicol assay has 53% cross-reactivity with thiamphenicol while the tylosin assay has 37% cross-reactivity with tilmicosin. The quinolone assay has 100% specificity for norfloxacin and lower specificities for the other drug compounds under the family. The streptomycin and ceftiofur assays have 100% specificity while the tetracycline assay has 100% specificity for tetracycline, around 50% specificity for oxytetracycline and chlortetracycline, and 12% specificity for doxycycline.

The results were multiplied with the dilution factor (20) to obtain the specific levels of residues per sample. Values were tabulated and compared with the established maximum residue limit (MRL) of the Codex Alimentarius Commission (CAC, 2018) for ceftiofur and the Commission Regulation (EU) No. 37/2010 for enrofloxacin, florfenicol, streptomycin, tylosin, and tetracycline.

RESULTS AND DISCUSSION

All samples tested positive for tetracycline and ceftiofur. Quinolone and florfenicol residues were detected in 45% and 5% of the samples, respectively, whereas streptomycin and tylosin were not detected at all (Table 1). Of the positive samples, tetracycline occurred at the highest concentration followed by ceftiofur. Quinolone residues occurred at relatively low levels while florfenicol had the least residue concentration. The residue levels did not exceed the established MRLs for finfish muscle (Table 2). A small percentage of samples had residues from two or four classes of antibacterial while more than half of the samples contained residues from three antibacterial classes (Table 3).

Tetracycline is the most commonly utilized antimicrobial in aquaculture (Done et al., 2015). It is also the only antimicrobial under the Philippine National Standards licensed for use in finfish (PNS, 2015). However, the occurrence of

<table>
<thead>
<tr>
<th>Fish source</th>
<th>n</th>
<th>QNL (%)</th>
<th>CEF (%)</th>
<th>FLO (%)</th>
<th>STR (%)</th>
<th>TYL (%)</th>
<th>TCT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Los Baños</td>
<td>54</td>
<td>(83.33)</td>
<td>(100)</td>
<td>(9.26)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Bayog</td>
<td>9</td>
<td>(44.44)</td>
<td>(100)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Mayondon</td>
<td>9</td>
<td>(100)</td>
<td>(100)</td>
<td>(11.11)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Malinta</td>
<td>9</td>
<td>(100)</td>
<td>(100)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Baybayin</td>
<td>9</td>
<td>(55.56)</td>
<td>(100)</td>
<td>(33.33)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Bambang</td>
<td>9</td>
<td>(100)</td>
<td>(100)</td>
<td>(22.22)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
<tr>
<td>Tadlac</td>
<td>9</td>
<td>(100)</td>
<td>(100)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(100)</td>
</tr>
</tbody>
</table>


% = (number of samples with the residue/total number of samples) x 100%.
Table 2. Mean antibiotic residue levels (μg/kg) in Nile tilapia meat samples from selected fish cages in coastal barangays of Los Baños.

<table>
<thead>
<tr>
<th>Fish source</th>
<th>n</th>
<th>QNL (100)</th>
<th>CEF (1000)</th>
<th>FLO (600)</th>
<th>STR (600)</th>
<th>TYL (100)</th>
<th>TCT (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayog</td>
<td>9</td>
<td>1.04±1.50</td>
<td>13.69±4.40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27.69±5.48</td>
</tr>
<tr>
<td>Mayondon</td>
<td>9</td>
<td>9.33±5.18</td>
<td>21.98±3.78</td>
<td>0.76±2.27</td>
<td>0</td>
<td>0</td>
<td>37.82±6.18</td>
</tr>
<tr>
<td>Malinta</td>
<td>9</td>
<td>9.18±2.69</td>
<td>26.69±3.68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>37.02±2.3</td>
</tr>
<tr>
<td>Baybayin</td>
<td>9</td>
<td>2.98±3.66</td>
<td>16.71±7.11</td>
<td>1.96±3.80</td>
<td>0</td>
<td>0</td>
<td>29.71±12.60</td>
</tr>
<tr>
<td>Bambang</td>
<td>9</td>
<td>9.33±5.18</td>
<td>16.44±4.80</td>
<td>0.84±1.69</td>
<td>0</td>
<td>0</td>
<td>36.98±13.27</td>
</tr>
<tr>
<td>Tadlac</td>
<td>9</td>
<td>8.93±5.97</td>
<td>16.62±6.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39.38±10.33</td>
</tr>
</tbody>
</table>

1^MRL permitted in seafood based on Commission Regulation (UE) NO 37/2010
2^MRL based on Codex Alimentarius Commission (CAC)

Table 3. Percentage of Nile tilapia meat samples with multi-drug residues in each coastal barangay of Los Baños.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Two drug residues (%)</th>
<th>Three drug residues (%)</th>
<th>Four drug residues (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayog</td>
<td>9</td>
<td>5 (55.56)</td>
<td>4 (44.44)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>Mayondon</td>
<td>9</td>
<td>0(-)</td>
<td>8 (88.89)</td>
<td>1 (11.11)</td>
</tr>
<tr>
<td>Malinta</td>
<td>9</td>
<td>0(-)</td>
<td>9 (100)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>Baybayin</td>
<td>9</td>
<td>3 (33.33)</td>
<td>5 (55.56)</td>
<td>1 (11.11)</td>
</tr>
<tr>
<td>Bambang</td>
<td>9</td>
<td>0(-)</td>
<td>7 (77.78)</td>
<td>2 (22.22)</td>
</tr>
<tr>
<td>Tadlac</td>
<td>9</td>
<td>0(-)</td>
<td>7 (77.78)</td>
<td>2 (22.22)</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>8 (14.81)</td>
<td>31 (57.41)</td>
<td>6 (11.11)</td>
</tr>
</tbody>
</table>

% = (number of samples with residues/ total number of samples) x 100%

this drug at lower than MRL in this study is not a guarantee of observance of proper dosing and withdrawal period as tetracyclines in general are poorly absorbed by fish (Ueno et al., 1995; Agwu and MacGowan, 2006). It could also be due to the leaching off of this antibacterial from fish feed. Other studies that demonstrated the presence of tetracycline residues in fish samples were those by Barani and Fallah (2014), Barman et al., (2018), Hossain et al. (2018), and Shim et al. (2010).

To our knowledge, this is the first report on the presence of ceftiofur residues in local finfish. It should be noted that the biochip array used in this study is highly specific to ceftiofur (Gaudin et al., 2016). As a veterinary drug, ceftiofur has only been approved for parenteral administration in livestock and in poultry thus, its use in fish would be considered extra-label. The possibility of ceftiofur residues originating from livestock farms cannot also be discounted. Although the biodegradation rate of ceftiofur has been shown to increase in wastewater from intensive livestock operations (Li et al., 2011), this might not be the case in farms directly
discharging effluent into the lake, which was confirmed as a manure management practice by some swine farm respondents in the province (Paraso et al., 2010).

Oxolinic acid is the most commonly used quinolone in Philippine aquaculture followed by flumequine and enrofloxacin (Alday-Sanz, 2012). It is the only quinolone used by milkfish and shrimp farmers and operators in a nationwide survey conducted by Cruz-Lacierda et al. (2000). Its residues in shrimp and milkfish samples have been demonstrated (Regidor and Somga, 2017). In South Korea, the most frequently detected veterinary drug residue in fish is quinolone, particularly enrofloxacin followed by ciprofloxacin, and oxolinic acid (Kang et al., 2018). Enrofloxacin was also the most frequently detected veterinary drug residue in fish samples in Vietnam (Uchida et al., 2016). Quinolones are very persistent in sediments, and detectable residues may remain for six months or more after drug treatment (Weston, 2000).

Florfenicol is a fluorinated derivative of thiamphenicol but is more commonly used than the latter since it shows an equal or superior effect in vitro against several pathogenic bacteria (Fodey et al., 2013; Ren et al., 2016). The pharmacokinetics of florfenicol in freshwater finfish such as crucian carp (Carassius auratus cuvieri) and Nile tilapia (Zaki et al., 2011) showed that it has excellent bioavailability, rapid absorption, and extensive distribution. It has substantial solubility in water but does not accumulate in biota or sediments. This is in accordance to the study of Zhong et al. (2010) where florfenicol was observed in water samples collected from coastal areas but not in sediment samples.

One of the significant findings in this study was the detection of multi-drug residues in all samples. The detected residues (ceftiofur, tetracycline, quinolone, florfenicol) are not only commonly used in aquaculture (Regidor and Somga, 2017), they also belong to critically important classes of antimicrobials in human medicine (FAO/WHO/OIE, 2006). The concurrent use of these antibiotics in fish is suggestive of irrational drug use. Another aspect that merits further investigation is the potential contamination of the lake water and sediments with the antibiotic residues detected in this study. Water samples from the lake previously showed levels of sulfonamides, trimethoprim, and lincomycin (Suzuki et al., 2013).

Low residue levels in food of animal origin can pose a health risk to consumers since chronic consumption can alter the intestinal ecology and favor the emergence of resistant bacterial pathogens (Cañada-Cañada et al., 2009), among others. This necessitates the implementation of an effective program on drug residue monitoring and surveillance as well as regulation in the use of antibiotics in cultured fish.

The results of this study showed that Nile tilapia cultured in Laguna de Bay along the coastal barangays of Los Baños contain antibiotic residues. Although the concentrations are lower than MRL, fish consumers can be exposed to the associated health risks. Future research should consider confirmatory testing to identify the specific antibiotics in the detected residues, and screening for antibiotic residues in fish samples from other sites of the lake. It is also worthwhile to determine the source of the residues, i.e. antibacterial use in aquaculture, or environmental contamination from human or livestock use.

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STATEMENT ON CONFLICT OF INTEREST

The authors have no competing interests to declare.

AUTHOR’S CONTRIBUTION

MGVP and MEPR conceptualized and designed the research, and drafted the manuscript. MEPR and SEAS processed and analyzed the samples, and collected the data. All authors read and approved the manuscript.

REFERENCES


Alday-Sanz V, Corsin F, Irde E and Bondad-Reantaso MG. 2012. Survey on the use of veterinary medicines in aquaculture. In: Improving biosecurity through prudent and responsible use of


