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Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States

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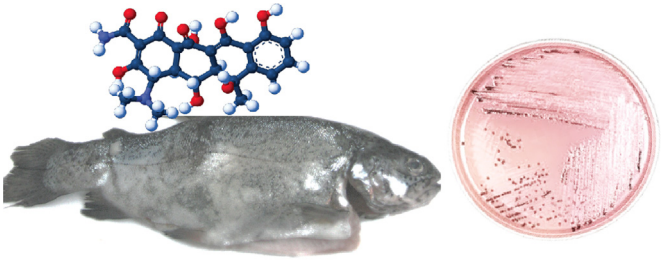
HIGHLIGHTS

- 5 out of 47 antibiotics were detected in shrimp, salmon, tilapia and trout.
- Oxytetracycline is the most commonly detected antibiotic compound.
- Publications reporting antibiotic resistance in aquaculture have increased 8-fold over 3 decades.
- We report a low risk of drug exposure from consumption of U.S. seafoods.
- We recommend vigilance toward stemming microbial risks.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquaculture production has nearly tripled in the last two decades, bringing with it a significant increase in the use of antibiotics. Using liquid chromatography/tandem mass spectrometry (LC–MS/MS), the presence of 47 antibiotics was investigated in U.S. purchased shrimp, salmon, catfish, trout, tilapia, and swai originating from 11 different countries. All samples (n = 27) complied with U.S. FDA regulations and five antibiotics were detected above the limits of detection: oxytetracycline (in wild shrimp, 7.7 ng/g of fresh weight; farmed tilapia, 2.7; farmed salmon, 8.6; farmed trout with spinal deformities, 3.9), 4-epioxytetracycline (farmed salmon, 4.1), sulfadimethoxine (farmed shrimp, 0.3), ormetoprim (farmed salmon, 0.5), and virginiamycin (farmed salmon marketed as antibiotic-free, 5.2). A literature review showed that sub-regulatory levels of antibiotics, as found here, can promote resistance development; publications linking aquaculture to this have increased more than 8-fold from 1991 to 2013. Although this study was limited in size and employed sample pooling, it represents the largest reconnaissance of antibiotics in U.S. seafood to date, providing data on previously unmonitored antibiotics and on farmed trout with spinal deformities. Results indicate low levels of antibiotic residues and general compliance with U.S. regulations. The potential for development of microbial drug resistance was identified as a key concern and research priority.

1. Introduction

It is estimated that within the next few years, aquaculture will account for almost 40% of total global seafood production by weight, up from 4% in 1970 [1,2]. This increase to a projected worldwide production of 83 million metric tons in 2013 has been due to a heightened demand for seafood, improved aquaculture techniques, emergence as a key cash crop in certain regions of the world, and recognition as a cheaper way to obtain high-quality protein [2,3]. However, as production surges,

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many aquaculture facilities resort to antibiotics to combat diseases in an environment that creates ample opportunities for bacterial pathogens to thrive [4]. Antibiotics are also commonly used as a prophylactic, sometimes on a daily basis [5]. Although some promising alternatives such as short-chain fatty acids and bacteriophage therapy have been proposed, many are not ready for mass usage [5]. Developed vaccines show promise in reducing antibiotic usage [4], but are only available to treat certain diseases and are not as cost-effective as antibiotics. Thus, the usage of antibiotics in aquaculture remains high.

Consequences associated with the use of antibiotics in aquaculture include the spread of antibiotics into the environment [6,7], residual concentrations left in seafood, high exposure by aquaculture facility personnel, and antibiotic resistance development [3,4]. Another issue is the impact of antibiotics on the animals themselves, such as potential changes in gene expression [8,9] and physiological anomalies. These physiological anomalies include malformation of the spine reported in fish exposed to oxytetracycline [10].

Many of the antibiotics used in aquaculture are also used in human medicine [11]. Amoxicillin and ampicillin are commonly prescribed for treating bacterial infections such as pneumonia and gastroenteritis [12]. As fish are a potential source of bacterial pathogens for humans, it is important to monitor the spread of antibiotic resistance amongst seafood [13]. Resistance to the most commonly applied antibiotics has been found in previous studies [3,14–16], including several that are multi-drug resistant (MDR) to many classes of antibiotics important in treating human infections [16–19]. Thus, detecting and monitoring antibiotic residues in seafood is critically important to reduce potential environmental and human health risks.

A large portion of aquaculture takes place in countries with few regulations and limited enforcement [20], creating the need to monitor imported seafood strictly for antibiotic residues and presence of pathogens. In this study, twenty-seven seafood samples were collected by the National Oceanic and Atmospheric Administration (NOAA) from stores in Arizona and California for analysis. Samples included five of the top ten most consumed seafood varieties in the U.S.: shrimp, tilapia, catfish, swai, and Atlantic salmon. Trout with visible deformed spines were also analyzed. Using liquid chromatography tandem mass spectrometry (LC–MS/MS), 47 antibiotics identified from literature as drugs of concern were analyzed for using two methods. We also conducted a meta-analysis of published data on antibiotics and resistance development to note trends in aquaculture over the last few decades.

2. Materials and methods

2.1. Samples and preparation

A collaborating NOAA consumer safety officer obtained samples ($n = 27$) from retail grocery stores in Arizona and California (in southwest U.S.) over a period of three months from June to August in 2012 (Table 1). Samples originated from 11 different countries. Each sample was sold as a pre-packed unit or bought from store counter displays, meaning that each sample sometimes included multiple fish. Negative controls consisted of catfish donated from Louisiana State University that were never exposed to antibiotics. Normal and deformed rainbow trout (n=3 for each) were obtained to survey the potential link between antibiotic exposure and spinal deformities. Atlantic salmon marketed as "antibiotic-free" was also obtained from a local health food store.

Whole fish were filleted and only edible parts were used for analysis. Shrimp ($n = 6$), tilapia ($n = 3$), catfish ($n = 5$), rainbow trout ($n = 6$), Atlantic salmon ($n = 5$), and swai ($n = 2$) were stored at −20 °C prior to processing by homogenization, using a commercial meat grinder (STX Turbo Force 3000 Series Electric Meat Grinder, Lincoln, Nebraska). Between processing of individual samples, the grinder was cleaned with warm water and soap, and then rinsed separately with acetone, ethanol, and distilled water three times each. Composite samples were prepared by pooling equal amounts of individual samples to result in 11 composite samples: farmed shrimp, wild-caught shrimp, farmed tilapia, farmed catfish, antibiotic-free catfish, farmed rainbow trout of normal habitus, farmed rainbow trout with deformed spine, farmed international Atlantic salmon, farmed antibiotic-free Atlantic salmon, farmed U.S. Atlantic salmon, and farmed swai (Table 1).

2.2. Sample analysis

Samples pre-processed as described above were frozen and shipped to a commercial laboratory (AXYS Analytical Services Ltd., Sydney, British Columbia, Canada). Approximately 2.5 g fresh weight (wet weight) of homogenized seafood was subsampled and spiked with isotope-labeled surrogates. Samples were then extracted by bath sonication with 15 mL acetonitrile that was acidified to pH 2 using 0.14 M NaH_2PO_4/85% H_3PO (1.93 g $NaH_2PO_4 \cdot H_2O$, 99 mL reagent water, 1 mL 85% H_3PO_4). The extract was then treated with 500 mg of solid ethylenediaminetetraacetic acid (EDTA). Resultant extracts were then filtered and cleaned using solid phase extraction (Waters Oasis HLB SPE cartridges 20 cm^3/1 g LP; Hartford, CT). For each sample, 30 mL of extract was diluted to 200 mL total volume with ultra pure water. Prior to sample loading, the cartridges were conditioned using 20 mL of methanol, 6 mL ultra pure water, and 6 mL pH 2 water. The cartridges were then washed with 10 mL of ultra pure water and subsequently dried under a vacuum. Analytes were eluted using 12 mL methanol, and the eluate concentrated under vacuum to a volume of 4 mL prior to analysis. The full 2.5 g of sample was extracted and contained in the final 4 mL extract.

Samples were analyzed by positive electrospray ionization on a triple quadrupole LC–MS/MS in multiple reaction monitoring (MRM) mode using a Waters Micromass Quattro Ultima LC-MS/MS System paired with a Waters LC 2795. Chromatography was conducted using reverse-phased C_{18} column (Waters, Milford, MA). A total of 60 pharmaceuticals were analyzed according to the AXYS Method MLA-075, a modification of the USEPA Method 1694 as described previously [21]. Out of the 60 analytes screened for, 47 were antibiotics, and are the focus of this paper (Table 2 and SI Table S1). Two methods were used on the same extract (injection volume: 10 uL) to analyze for tetracyclines and non-tetracyclines, respectively. The tetracyclines method, totaling 30 min in duration, had solvent A consisting of an equal mixture of acetonitrile and methanol with 0.5 mM oxalic acid and 0.5% (v/v) formic acid; solvent B consisted of HPLC-grade water containing 0.5 mM oxalic acid and 0.5% (v/v) formic acid. The starting mixture was 10% solvent A (flow rate 0.2 mL/min), increased to 90% A by minute 20 at a flow rate of 0.23 mL/min. The non-tetracyclines method had a run time of 33 min, using as solvent A HPLC-grade water with 0.1% formic acid and 0.1% ammonium formate, and as solvent B a mixture of equal amounts of acetonitrile and methanol. The starting mixture was 95% solvent A (flow rate 0.15 mL/min), increased to 100% solvent B by minute 23 at a flow rate 0.3 mL/min. For the 10 of the 60 total compounds for which a respective stable-isotope labeled analog was available, the concentration was determined using the isotope dilution technique [22]. For the remaining 50 compounds where a labeled analog was not available, the concentration was determined using an alternate isotope-labeled internal standard (see supplemental information).

Precision between intraday samples and duplicates was expressed as relative percent difference (RPD), which was calculated using the following expression as reported previously [23]:

$$\text{RPD} \quad [\%] = \frac{|C_{\text{sample}} - C_{\text{duplicate}}| \times 100}{(C_{\text{sample}} + C_{\text{duplicate}})/2} \tag{1}$$

where C_{sample} and $C_{\text{duplicate}}$ are the concentrations detected in the original sample and in its duplicate, respectively.

2.3. Quality assurance and control

Several tests were performed before and during sample analysis to ensure system and laboratory performance. Initial calibration was performed using labeled surrogates, recovery standards and authentic targets to encompass the working concentration range. Retention times of native and labeled compounds had to be within 0.4 min of the respective retention time established during the previous calibration. A mid-level solution was analyzed every