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Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk

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ABSTRACT

Background: Antibiotics used globally to treat human and animal diseases exist ubiquitously in the environment at low doses because of misuse, overdose and poor absorption after ingestion, coupled with their high-water solubility and degradation resistance. However, the systemic chronic effects of exposure to low environmental concentrations of antibiotics (LECAs) and legal aquaculture doses of antibiotics (LADAs) in fish and their human health risk are currently unknown.
Objective: To investigate the in vivo chronic effects of exposure to LECAs and LADAs using oxytetracycline (OTC) and sulfamethoxazole (SMZ) in Nile tilapia (*Oreochromis niloticus*) and their human health risk.
Methods: Twenty *O. niloticus* weighing 27.73 ± 0.81 g were exposed to water containing LECAs (OTC at 420 ng/L and SMZ at 260 ng/L) and diets supplemented with LADAs (OTC 80 mg/kg/day and SMZ 100 mg/kg/day) for twelve weeks. General physiological functions, metabolic activities, intestinal and hepatic health were systemically evaluated. The possible human health risks of the consumption of the experimental Nile tilapia fillets in adults and children were assessed by using risk quotient.
Results: After exposure, we observed retarded growth performance accompanied by reduced nutrients digestibility, feed efficiency, organ indices, and lipid body composition in treated fish. Antibiotics distorted intestinal morphological features subsequently induced microbiota dysbiosis and suppressed intestinal tight junction proteins. Exposure of fish to LECAs and LADAs induced oxidative stress, suppressed innate immunity, stimulated inflammatory and detoxification responses, concomitantly inhibited antioxidant capacity and caused lipid peroxidation in intestine and liver organs. Both LECAs and LADAs enhanced gluconeogenesis, inhibited lipogenesis and fatty acid beta oxidation in intestine and liver organs. The exposure of fish to LECAs and LADAs induced anaerobic glycolytic pathway and affected intestinal fat catabolism in intestine while halted aerobic glycolysis, increased hepatic fat catabolism, and induced DNA damage in liver. The hazard risk quotient in children for fish treated with OTCD was > 1 indicating human health risk.
Conclusion: Overall, both LECAs and LADAs impair general physiological functions, nutritional metabolism, and compromise fish immune system. Consumption of fish fed with legal OTC provokes health risk in children. Global stringent prohibition policy for use of antibiotics in aquaculture production and strategies to limit their release into the environment are urgently required to protect human health.

1. Introduction

Antibiotics misuse, overdose and poor absorption after medication, coupled with their high-water solubility, bioactivity, and persistent behavior represent a global environment pollution pandemic that has caused severe ecological sustainability and health problems (Brandt et al., 2015; Carvalho and Santos, 2016), particularly in fish, which plays a central role in human food safety, mainly through consumption. Globally, antibiotics consumption increased from 50 to 70 billion standard units in 2000 to 2010 (Van Boeckel et al., 2014), with 63 tons consumed by livestock in 2010 (Van Boeckel et al., 2015), and projection indicates up to 105 tons are expected to be consumed by 2030

Abbreviations: LECAs, low environmental concentrations of antibiotics; LADAs, legal aquaculture doses of antibiotics; OTCD, oxytetracycline diet; SMZD, sulfamethoxazole diet; OTCB, oxytetracycline bath; SMZB, sulfamethoxazole bath; LEC-OTC/SMZ, low environmental concentrations of OTC and SMZ; LAD-OTC/SMZ, legal aquaculture doses of OTC and SMZ

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(CDDEP, 2015). Antibiotics used in various human activities are released into the aquatic environment where they exist at low concentrations (Sotto et al., 2017). High concentrations of antibiotics existing in the environment provoke fish population disturbances and endanger species extinction (Sanchez et al., 2011), while those used for treatment of bacterial diseases in various fish species in aquaculture production result into controversial outcomes on growth performance (Reda et al., 2013; Gaikowski et al., 2015; Zhang et al., 2015), cause body malformation (Zhang et al., 2016), microbiota dysfunction (Navarrete et al., 2008; He et al., 2012), and suppress immunity (Guardiola et al., 2012; Han et al., 2014). Moreover, antibiotics used in aquaculture induce oxidative stress (Nunes et al., 2015; Wang et al., 2016), affect antioxidant capacity (Liu et al., 2015), and trigger DNA damage (Botelho et al., 2015). However, fish cannot avoid exposure to antibiotics because of 1) legal dietary medication to prevent and cure diseases (Dobšíková et al., 2013; Phu et al., 2015) and 2) existence of low environmental residues in aquatic environments (Sotto et al., 2017). Despite this, the potential chronic effects of low environmental concentrations of antibiotics (LECAs) and their legal aquaculture doses (LADAs) in fish and their human health have not been thoroughly evaluated.

Oxytetracycline (OTC) and sulfamethoxazole (SMZ) are bacteriostatic antibiotics, which limit bacteria growth by interfering with protein synthesis, for which OTC binds to 30S ribosomal subunits and prevents ammonia acyl tRNA from combining with ribosome (Zhang et al., 2015), while SMZ blocks dihydrofolate intermediate production, thereby restricts the normal bacterial folic acid synthesis (Johansson et al., 2014). Both OTC and SMZ are used for treatments of bacterial diseases in cultured fish species due to their broad-spectrum bacteria activities, cost effectiveness and legal availability (Navarrete et al., 2008; Dobšíková et al., 2013; Phu et al., 2015). The OTC is administered in feeds at 50 to 250 mg/kg fish body weight/day for 3 to 21 days (Gaikowski et al., 2003; Navarrete et al., 2008; Yonar et al., 2011), while SMZ is commonly administered at 100 to 200 mg/kg fish body weight/day for 5 days (Liu et al., 2017), depending on fish species, infection and country-specific legal requirements. Despite these legal directives, the two antibiotics are used for long periods in aquaculture production (Phu et al., 2015), sometimes on daily basis and in absence of diseases (Pham et al., 2015).

Previous studies have reported that, OTC and SMZ are poorly absorbed in the guts of animals after medication, subsequently are excreted in urine and feces, either unchanged or modified into metabolites, which are transported into surface waters through runoff and subsurface drainage systems (Zhang et al., 2016). Thus, they exist omnipresent in the aquatic environments at concentrations ranging from 174.9 ± 266.9 ng/L to 741.85 ng/L in freshwater (Bai et al., 2014; Dong et al., 2016) and 15,163 ng/L in sea water for OTC (Chen et al., 2015), whereas SMZ have been detected in freshwater at concentrations ranging from 259.60 ng/L to 385.00 ng/L (Chen and Zhou, 2014; Y. Li et al., 2016). These concentrations pose risks on aquatic species, raising global public concern on human health upon fish consumption (Johansson et al., 2014; Botelho et al., 2015; Yan et al., 2016; Chen et al., 2017). However, no study has extensively explored the chronic effects of LECAs and LADAs, particularly using OTC and SMZ in fish and their human health effects.

In this study, we subjected fish to low concentrations of antibiotics existing in the aquatic environment and their legal doses used in aquaculture. For this purpose, we exposed Nile tilapia (*Oreochromis niloticus*), a global economic, cultured and consumed species, to dietary and bath treatments containing OTC and SMZ for twelve weeks. The aim was to explore the chronic systemic effects of LEC-OTC/SMZ and LAD-OTC/SMZ on general body functions and intestinal and hepatic health, which are important organs in fish and assess their human health risks after fish consumption. Although we only exposed fish using two methods and explored two antibiotics, we anticipate that the strategy used could be applied extensively in the assessments of chronic effects of other antibiotics and veterinary drugs in fish and human health risks.

2. Materials and methods

2.1. Antibiotics and fish exposure

Sulfamethoxazole (SMZ, CAS: 723-46-6) and oxytetracycline (OTC, CAS: 615 3-64-6) were purchased from Yuanmu Biotechnology Co., Ltd., Shanghai, China. About 200 all-male *O. niloticus* fingerlings were purchased from Shanghai Ocean University (Shanghai, China). All male *O. niloticus* were used for the experiment because they grow faster and at a uniform size than the females, thus, many farmers world-wide are more likely to culture them in the practical aquaculture. The fish were acclimatized in four aseptic 200-litre tanks (50 fish each), supplied with dechlorinated water for two weeks. Fish in each tank were supplied with compressed air via air-stones from air pumps at a 10 h/14 h light/dark cycle and water temperature, dissolved oxygen, pH and total ammonia nitrogen were maintained at ranges from 26 to 28 °C, 4.8 to 6.4 mg/L, 7.5 to 7.9 and < 0.02 mg/L, respectively. During this period, the fish were hand-fed using a commercial diet (Chengdu, China) containing ≥33% protein and ≥5% lipid. After acclimatization, all *O. niloticus* were individually embedded with a tracking chip, which aided individual fish recognition through radio-frequency identification (RFID) technology (Voulodimos et al., 2010) and fish were randomly distributed into five sterile 220-litre tanks (30 fish per tank) filled with dechlorinated water to a volume of 130 L/tank and allowed to settle for one week.

Twenty-four hours before the experiment, total initial mean weights of fish were determined as 27.73 ± 0.81 g for all treatments. In one pair of the experiment, *O. niloticus* were exposed to LADAs in feeds supplemented with 2.00 g/kg diet of OTC (80 mg/kg body weight/day) and 2.50 g/kg diet of SMZ (100 mg/kg body weight/day), hereafter, OTCD and SMZD, respectively; Table S1. Another pair of fish were exposed to LECAs (bath treatments, referred to as OTCB and SMZB, respectively) containing similar types of antibiotics at concentrations of 420 ng/L for OTCB and 260 ng/L for SMZB to reflect low environmental relevant exposure situations (Bai et al., 2014; Chen and Zhou, 2014). A control tank was included in which fish were reared and treated similarly but deprived of antibiotics. The experimental conditions were similar to those used during the acclimatization period. All fish were hand-fed twice daily between 9:00 and 10:00 h and 17:00 and 18:00 h at 4% of their average body weight per day for 84 days and the feeds contained 0.10 g/kg/diet yttrium oxide (Yt_2O_3) marker for estimation of apparent nutrients digestibility (Table S1). Fish in the diet treatments were fed by using little amounts of feeds gradually until all the feeds per feeding ration were consumed completely without much dissolution of antibiotics from the diets. Fish in the control and bath treatments were hand-fed on the basal diet (Table S1). Half of the water in the dietary and control tanks were replaced with flesh-dechlorinated water every 24 h, while those in bath treatments – antibiotics were replaced with flesh-dechlorinated water containing the initial concentrations of antibiotics in order to maintain relatively stable concentrations as designed. The weight of individual fish was recorded fortnightly and the feed rations were adjusted accordingly.

2.2. Growth performance, survival rate, feed efficiency and organ indices

At the end of the experiment, all survived fish were fasted for 24 h, they were collected by using a scoop net and their individual weights determined for weight gain and final weight estimations. Any death of fish during the experiment was recorded for survival rate determination. The amount of feed and the weight of fish were used to calculate feed efficiency as the ratio of total wet weight gained by fish to total amount of feed fed in each treatment.