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# A global analysis on the systemic effects of antibiotics in cultured fish and their potential human health risk: a review

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## Abstract

The rearing of fish in intensive systems reduces their immunity leading to eruption of diseases, consequently prompting the use of antibiotics. Antibiotics are currently used in cultured fish globally to treat/prevent diseases. On the one hand, antibiotics used in aquaculture help to improve fish health by killing or inhibiting pathogenic bacteria proliferation. However, on the other hand, they cause multiple side effects. To date, information on systemic effects of antibiotics in cultured fish is scattered and their potential human health risk remains elusive. This review critically analyses and discusses the available information in the literature on the positive and negative effects of antibiotics in cultured fish and their potential human health risk via fish consumption. The review found 44 different antibiotics dominated by oxytetracycline were studied in various fish species worldwide. Antibiotics used in global aquaculture either decrease or increase fish growth and survival rates. They induce oxidative stress, which affect antioxidant and detoxification responses and cause host–microbiota dysbiosis. These changes compromise the fish immunity system, which ultimately lead to cellular damage. The toxicities generated inhibit the fish aerobic glycolysis, suppress lipogenesis and fatty acid β-oxidation while increase energy demand by initiating gluconeogenesis and anaerobic glycolysis. Consumption of fish treated with antibiotics causes human health risk. Future studies are required on agents to restore dysfunctions induced by antibiotics in cultured fish, while attempts to limit their utilisation in aquaculture production are underway. [Correction added on 21 November 2020, after first online publication: The abstract has been amended to clarify the scope of the review.]

**Key words:** antibiotics, growth performance, human health risk, immunity, microbiota, oxidative stress.

## Introduction

Aquaculture has remained the fastest growing food production sector for the last 40 (1980–2020) years, currently supplying half of the fish consumed by humans globally (Troell *et al.* 2014; Golden *et al.* 2017). The increasing demand for aquaculture products has led to the development of intensive culture systems. However, the culture of fish under intensive production systems weakens their immunity, consequently leading to eruption of infectious diseases, which reduce growth, survival rate and cause heavy economic losses, that ultimately lower fish production. Accordingly, fish practitioners use antibiotics for prophylactic, metaphylactic and therapeutic purposes in order to avoid production loss and improve health (Cabello *et al.* 2013). Antibiotics used in global aquaculture fish production are regarded as a double-edged sword. On the one edge, they improve fish health by killing or inhibiting pathogenic bacteria proliferation, while on the other edge, they cause multiple side effects including biological, antibiotic resistance, environmental such as persistence of antibiotics in sediments, their ecological consequences in the water column and uptake by biota as well as consumer health risks.

Previous on-field and experimental studies on antibiotics used in aquaculture provided useful information on their effects in cultured fish (Rico *et al.* 2014; Rico & Van den Brink 2014). To date, several studies have indicated multiple effects of antibiotics in various fish species, including decreased (Gaikowski *et al.* 2015; Zhang *et al.* 2015; Limbu *et al.* 2018; Limbu *et al.* 2019; Limbu *et al.* 2020) and

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increased growth performance (Sanchez-Martínez *et al.* 2008; He *et al.* 2011a; Di Salvo *et al.* 2013; Reda *et al.* 2013; Zhou *et al.* 2018a). Other studies have reported body malformation (Zhang *et al.* 2016), microbiota dysfunction (He *et al.* 2012; Limbu *et al.* 2018; Zhou *et al.* 2018a; Zhou *et al.* 2018b; Limbu *et al.* 2019; Limbu *et al.* 2020) and immunity suppression (Guardiola *et al.* 2012; Han *et al.* 2014; Limbu *et al.* 2018; Limbu *et al.* 2020) in various fish species. Moreover, antibiotics induced oxidative stress (Nunes *et al.* 2015; Wang *et al.* 2016; Limbu *et al.* 2018; Zhou *et al.* 2018a; Zhou *et al.* 2018b; Limbu *et al.* 2019; Limbu *et al.* 2020), affected antioxidant capacity (Liu *et al.* 2015; Limbu *et al.* 2019) and caused nephrotoxicity (Hentschel *et al.* 2005) and DNA damage (Botelho *et al.* 2015; Limbu *et al.* 2019) in several fish species. Recently, direct human health risk linked to consumption of fish treated with antibiotics was reported in children (Limbu *et al.* 2018).

Despite these results, previous reviews on antibiotics used in aquaculture focused on antibiotic resistance (Alderman & Hastings 1998; Armstrong *et al.* 2005; Cabello *et al.* 2013; Tuševljak *et al.* 2013; Santos & Ramos 2018) and environmental risks (Armstrong *et al.* 2005; Burridge *et al.* 2010; Rico *et al.* 2012; Lulijwa *et al.* 2019). The potential human health risks via residues of antibiotics have also been reviewed (Cabello 2004; Sapkota *et al.* 2008; Heuer *et al.* 2009; Marshall & Levy 2011; Millanao *et al.* 2011; Cabello *et al.* 2013; Liu *et al.* 2017; Mo *et al.* 2017). Furthermore, policies and regulation of antibiotics use in the top 15 major aquaculture producers were reviewed by Lulijwa *et al.* (2019). For individual antibiotics, the pharmacokinetics, pharmacodynamics, dose optimization and withdrawal of oxytetracycline were reviewed by Rigos and Smith (2015). Currently, review studies focusing specifically on systemic effects of antibiotics on cultured fish, as an important and complicated topic and direct human health risk caused by consumption of fish treated with antibiotics, in global aquaculture, are limited. A recent publication reported the potential risks of antibiotics in fish and human health for the African continent (Limbu 2020). However, the study did not provide conclusive remarks, due to limited studies on antibiotics use in cultured fish in the continent (Limbu 2020). Thus, knowledge on the systemic effects of antibiotics used in cultured fish globally is currently unfocussed and scattered. Moreover, our understanding on human health risk resulting from consumption of fish treated with antibiotics is still limited.

For the first time, this review analyses the available information in the literature on both the positive and negative effects of antibiotics used in global cultured fish. First, we discuss the effects of antibiotics on growth performance, feed utilization, microbiota, immunity, toxicities and detoxification mechanisms in cultured fish. Second, we examine the potential human health risk resulting from direct consumption of fish treated with antibiotics. Finally, we close our review by providing concluding remarks and suggesting future perspectives. The information generated is critical to global fish aquaculture practitioners as a tool to evaluate comprehensively the side effects caused by antibiotics, considering that the European Union moratorium (Regulation (EC) No. 1831/2003) banned their use as feed additives in animal feeds (Regulation 2003) and the current public criticisms on their use in fish production worldwide due to increased resistance bacteria and genes.

## Current common list of antibiotics used in global aquaculture production

It is globally agreed that, all antibiotics legally used in aquaculture must be approved by the government agency responsible for veterinary medicine (Romero *et al.* 2012). However, little information exists on antibiotics used in aquaculture because many regulatory agencies have failed to collect the information and/or absence/weak regulations concerning their use (Burridge *et al.* 2010; Marshall & Levy 2011), coupled with diverse distributions and registration systems in different countries (Romero *et al.* 2012). Moreover, the regulating agencies usually set doses and withdrawal periods for various antibiotics in different fish species. Unfortunately, antibiotics are administered abusively, for longer periods due to lack of knowledge about their purpose and proper application by producers (Pham *et al.* 2015) and the lack of legislation, enforcement and inspection by regulatory agencies. Normally, antibiotics are applied in fish on a daily basis and in the absence of disease problems (Holmström *et al.* 2003; Defoirdt *et al.* 2011; Pham *et al.* 2015; Phu *et al.* 2015) to health, carrier and diseased fish (Cabello *et al.* 2013).

Based on country-specific regulations, certain types of antibiotics are permitted for use in aquaculture production (see Table 1). The common antibiotic groups permitted in major aquaculture-producing countries have changed significantly over the years, due to the banning of several antibiotics (Alderman & Hastings 1998; Armstrong *et al.* 2005; Heuer *et al.* 2009; Broughton & Walker 2010; Burridge *et al.* 2010; Defoirdt *et al.* 2011; Rico *et al.* 2012; Romero *et al.* 2012; Cabello *et al.* 2013; Mo *et al.* 2017). We wish to inform that, the common antibiotics used in aquaculture is a broad topic, beyond the objective of the current review, and such a subject matter has already been reviewed (Alderman & Hastings 1998; Holmström *et al.* 2003; Cabello 2004; Armstrong *et al.* 2005; Cabello 2006; Sapkota *et al.* 2008; Heuer *et al.* 2009; Burridge *et al.* 2010; Defoirdt *et al.* 2011; Marshall & Levy 2011; Millanao *et al.* 2011; Rico *et al.* 2012; Romero *et al.* 2012; Cabello *et al.* 2013; Rico *et al.* 2013; Liu *et al.* 2017; Mo *et al.* 2017).

The types of antibiotics used in aquaculture vary markedly among countries. In some countries, regulations on antibiotics used for aquaculture production are enforced



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**Table 1** Common antibiotics permitted and used in aquaculture in various countries

| Category | Antibiotic | Permitted[†] | Country reported VT PH | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TH | CH | IT | CN | VT | PH |
| Aminoglycosides | Streptomycin | NOI | | √ | | √ | | |
| | Gentamycin | NOI | | √ | | √ | | √ |
| | Kanamycin | NOI | | √ | | | | |
| | Neomycin | Yes | | | | √ | | |
| Amphenicols | Florfenicol | Yes | | √ | | √ | √ | |
| | Chloramphenicol | NOI | | √ | | √ | | √ |
| | Thiamphenicol | Yes | | | √ | | | |
| β-lactams | Amoxicillin | Yes | | √ | √ | √ | √ | |
| | Ampicillin | NOI | | | | | √ | |
| | Benzyl penicillin | NOI | | | | √ | | |
| | Penicillin | NOI | | | | √ | | |
| Fluoroquinolones | Ciprofloxacin | NOI | | | | √ | | √ |
| | Enrofloxacin | Yes | √ | √ | | √ | √ | √ |
| | Ofloxacin | NOI | | | | | | |
| | Norfloxacin | Yes | √ | √ | | √ | | √ |
| | Oxolinic acid | Yes | | √ | | √ | | √ |
| | Pefloxacin | NOI | | | | | | |
| | Flumequine | Yes | | √ | √ | | | |
| | Nalidixic acid | NOI | | √ | | | | |
| | Piromidic acid | NOI | | | | | | |
| Cephalosporin | Cefotaxime | NOI | | √ | | | | |
| Macrolides | Erythromycin | NOI | | √ | | √ | | √ |
| Nitrofurans | Furazolidone | NOI | | √ | | √ | | |
| | Nitrofurazone | NOI | | | | | | √ |
| Nitroimidazoles | Metronidazole | NOI | | | | | √ | |
| Polymyxins | Colistin | NOI | | | | | √ | |
| Rifamycins | Rifampicin | NOI | | | | √ | | |
| Sulphonamides | Sulfadimethoxine/ormetoprim | Yes | √ | | | | | |
| | Sulfamethoxazole | Yes | | | | √ | √ | |
| | Sulfamonomethoxine | Yes | | | | √ | | √ |
| | Sulfamethazine | Yes | | | | | | √ |
| | Sulfisoxazole | NOI | | | | √ | | |
| | Sulfamerazine | NOI | | | √ | | | |
| | Trimethoprim | Yes | | √ | | | √ | √ |
| | Sulfadiazine | Yes | | | | √ | √ | |
| Tetracyclines | Oxytetracycline | Yes | √ | | | √ | √ | √ |
| | Chlortetracycline | NOI | | | | √ | | √ |
| | Tetracycline | NOI | | √ | √ | | | √ |
| | Doxycycline | Yes | | | | √ | √ | |

CH, Chile; CN, China; IT, Italy; PH, Philippines; TH, Thailand; VT, Vietnam.

NOI means no information.

[†]Indicates antibiotics permitted in Chinese aquaculture while √reflects reported antibiotic.

strictly, such that only a few antibiotics are licensed. For example, in the United States of America (USA), only three antibiotics [oxytetracycline (OTC), florfenicol (FFC) and sulfadimethoxine/ormetoprim (SDM/OMP)] are permitted for use in aquaculture in an attempt to protect cultured fish health and consumer safety (U.S. Department of Health & Human Services 2008). Similarly, the United Kingdom (UK) permits only three antibiotics for treating diseased fish in aquaculture, namely OTC, FFC and amoxicillin (AMX), with suppliers identified (Veterinary Medicines Directorate (GB) 2017). In Norway, three antibiotics [FFC, flumequine (FMQ) and oxolinic acid (OA)] are licensed for use in farmed fish (Midtlyng *et al.* 2011). In Canada, four antibiotics [OTC, FFC, SDM/OMP and sulfadiazine/trimethoprim (SD/TMP)] are approved by the Veterinary Drugs Directorate (VDD) of Health Canada to treat infected fish (Trudel *et al.* 2016).

Nevertheless, a great quantity of global aquaculture production occurs in countries, which permit the use of several


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antibiotics. For instance, China, the current largest producer and consumer of aquaculture products, permits the use of 12 different antibiotics (Liu *et al.* 2017). Chile, one of the major aquaculture producers (FAO 2018), permits chloramphenicol (CAP), gentamycin and furazolidone (Cabello *et al.* 2013), in addition to common antibiotics used in other countries. In India, another major aquaculture producer, permits SD/TMP, chlortetracycline, doxycycline (DOX), nitrofurazone/furazolidone, CAP and neomycin/DOX (Pathak *et al.* 2000). However, during the review period, it was difficult to obtain the information on current antibiotics permitted in different countries due to the reasons explained at the beginning of this section (Table 1).

Country-specific studies on common antibiotics used in aquaculture globally suggest that, more antibiotics are administered abusively than those permitted (Table 1). Most antibiotics are administered on fish through medicated feeds and bath treatments (Heuer *et al.* 2009; Romero *et al.* 2012). Previous studies reported at least 13 different antibiotics were used in Thailand (Holmström *et al.* 2003) and Chile (Cabello 2004; Millanao *et al.* 2011), 5 in Italy, (Lalumera *et al.* 2004) and 14 in Vietnam (Nguyen *et al.* 2015) (Table 1). In China, 20 different antibiotics were reported in aquaculture instead of 12 permitted, reflecting misuse of antibiotics (Liu *et al.* 2017). Moreover, CAP, erythromycin (ERY), furazolidone, ciprofloxacin (CPF) and norfloxacin (NOR) were detected in China despite their ban since 2002 and 2016, respectively (Liu *et al.* 2017), highlighting abusive use of antibiotics in aquaculture. In the USA, four antibiotic compounds were detected in water from 13 fish hatcheries, including tetracycline (TC) (Dietze *et al.* 2005), which is not registered for use in aquaculture, suggesting, trace impurities from OTC or its transformation or it might be used illegally.

Multi-country studies also indicate variations in the number of antibiotics used, which are mostly administered abusively, although the number of antibiotics groups applied is relatively similar. Rico *et al.* (2013) revealed OTC, AMX, FFC and NOR were reported in at least two countries among China, Thailand and Vietnam, therein each country used a maximum of two antibiotics on any individual farm. Conversely, Pangasius farms in Vietnam applied 17 distinct antibiotics categorized into 10 different classes (Rico *et al.* 2013). More multi-country studies were conducted by Sapkota *et al.* (2008) and Lulijwa *et al.* (2019) in 15 aquaculture-producing countries, Rico *et al.* (2012) in seven Asian major aquaculture-producing countries and Tuševljak *et al.* (2013) for the global aquaculture production. In these studies, Sapkota *et al.* (2008) reported 26 different antibiotics (categorized into eight groups), Rico *et al.* (2012) found 36 antibiotics (classified into eight groups), and Tuševljak *et al.* (2013) revealed eight antibiotics groups. In all these studies, antibiotics detected were dominated by OTC. China ranked second in the number of different types of antibiotics used (17 types), with Vietnam recording the highest number (31 distinct types) (Rico *et al.* 2012). Furthermore, Vietnam was the only country, which used metronidazole (MTZ), colistin and FFC in aquaculture production. On the other hand, Rifampicin (Rifamycins) was the only antibiotic used by China and Philippines. Similarly, the use of quinolone was reported as the most 'frequent-to-almost always used antibiotic' by 70% and 67% of respondents from the USA and Canada, respectively (Tuševljak *et al.* 2013). This is unusual because quinolone products are not approved for aquaculture and fluoroquinolone use is extra-labelled either prohibited (USA) or discouraged (Canada). The majority of respondents also reported similar frequencies of quinolone use in aquaculture from Europe (70%) and Asia (90%), where labelled prohibition indications exist (Tuševljak *et al.* 2013). More recently, Lulijwa *et al.* (2019) found 67 antibiotic compounds were used in 11 of the 15 aquaculture-producing countries for all cultured species and their environment. These studies suggest abusive use of antibiotics in global aquaculture production.

In short, clear variations exist in regulations and control of antibiotics used in global cultured fish species. Aquaculture currently uses at least eight different common groups of antibiotics (dominated by tetracyclines particularly OTC), which are mostly used abusively. The various antibiotics induce distinct systemic effects in fish body, owing to their binding behaviour on different biochemical components (Chi *et al.* 2011; Andrieu *et al.* 2015), as detailed in the next sections.

## Systemic effects of antibiotics on cultured fish

The application of antibiotics in aquaculture, for fish health management, has become an integral part of the industry (Limbu 2020). Antibiotics are absorbed directly into the gastrointestinal tract in fish body following their ingestion, where they accumulate and/or distribute into different tissues depending on their mode and site of actions. Accordingly, antibiotics cause multiple effects in fish than their primary purpose, depending on species and their growth stage, antibiotics types and doses and mode of action. The effects induced by antibiotics are most likely to affect the overall aquaculture production of fish as detailed in the subsequent sections.

## Growth performance and feed utilization

Growth performance and feed utilization are important production attributes to fish growers, because they affect directly the yield and economics of aquaculture enterprises. Thus, understanding the effects of antibiotics on these



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aspects in cultured fish deserves a peculiar consideration. Our review revealed different antibiotics dominated by OTC were studied in various fish species worldwide, to evaluate growth and other effects (Table 2).

Most of the available reports on the effects of antibiotics on fish growth performance indicate inconsistent results, both for dietary and bath treatments (Table 2). Accordingly, various doses of dietary antibiotics did not affect growth performance in many fish species (Wagner 1954; Snieszko & Wood 1955; Robinson *et al.* 1990; Hustvedt *et al.* 1991; Inglis *et al.* 1991; Rawles *et al.* 1997; Gaikowski *et al.* 2003a; Gaikowski *et al.* 2003b; Refstie *et al.* 2006; Zhou *et al.* 2009; Straus *et al.* 2012; Dobšíková *et al.* 2013; Limbu *et al.* 2018; Nakano *et al.* 2018; Zhou *et al.* 2018a), similar to bath exposure (Bartoskova *et al.,* 2013; Plhalova *et al.* 2014; Fonte *et al.* 2016; Yan *et al.* 2016; Zhou *et al.* 2018b). The comparable growth obtained is attributed to the lack of initial diverse microflora communities in fish (Rawles *et al.* 1997), undisrupted nutrient absorption mechanisms (Refstie *et al.* 2006), existence of similar appetite and feed consumption (Inglis *et al.* 1991; Gaikowski *et al.* 2003a; Zhou *et al.* 2009) and feed efficiency (Gaikowski *et al.* 2003b).

On the contrary, many lines of evidences exist on the reduced growth performance in various fish species fed on several dietary doses of therapeutic (Boujard *et al.* 1997; Gaikowski *et al.* 2003a; Rábago-Castro *et al.* 2006; Gaikowski *et al.* 2013; Gaikowski *et al.* 2015; Limbu *et al.* 2018; Limbu *et al.* 2019; Limbu *et al.* 2020), excessive (Snieszko & Wood 1955) and different levels (Manning *et al.* 2013) of antibiotics, based on dose (Gaikowski *et al.* 2003a; Manning *et al.* 2013) and fish species (Snieszko & Wood 1955). Similarly, bath exposure to antibiotics at environmental relevant levels (Yan *et al.* 2016; Qiu *et al.* 2020; Liu *et al.* 2020b) and toxicological concentrations (Zhang *et al.* 2015) reduced growth in various fish species, depending on dose (Zhang *et al.* 2015; Yan *et al.* 2016) and antibiotic types (Yan *et al.* 2016). Decreased growth performance, after both dietary and bath exposure, has been attributed to reduced appetite, voluntary feed intake, feed efficiency, protein efficiency ratio, feed consumption and feed palatability (Snieszko & Wood 1955; Boujard *et al.* 1997; Rábago-Castro *et al.* 2006; Gaikowski *et al.* 2013; Manning *et al.* 2013; Limbu *et al.* 2019; Limbu *et al.* 2020), coupled with antibiotics mediated toxicity, which cause nutrients malabsorption (Zhang *et al.* 2015). However, these reasons do not explain universally all the obtained results in cultured fish. For instance, although growth was suppressed in *I. punctatus* treated with SDM/OMP, feeding appetite was not affected (Rábago-Castro *et al.* 2006), similar to *M. saxatilis* × *M. chrysops* hybrids and *O. niloticus* treated with OTC and FFC, respectively, where feed consumption was not affected (Gaikowski *et al.* 2003b; Gaikowski *et al.* 2015). In contrast, *I. punctatus* treated with FFC and *O. mykiss* fed on OTC had similar growth rate, although feed intake was reduced (Hustvedt *et al.* 1991; Rawles *et al.* 1997). These results suggest other reasons are responsible for reduced growth in fish after antibiotics administration.

The reduced growth of cultured fish after antibiotics is explained partly by impaired digestive enzymes activities and nutrients digestibility. Indeed, dietary and bath treatments using OTC and sulfamethoxazole (SMZ) reduced protease and lipase activities, together with protein and lipid digestibility in *O. niloticus* after chronic exposure for 12 weeks (Limbu *et al.* 2018). However, dietary OTC treatment did not affect trypsin and amylase activities in *S. salar* after three weeks (Refstie *et al.* 2006) and dietary OTC and SMZ increased amylase and lipase activities in *D. rerio* after six weeks (Zhou *et al.* 2018a). These results suggest that, antibiotics affect enzymes activities of cultured fish depending on species and time of exposure. We hypothesize that, antibiotics bind and aggregate directly with digestive enzymes (Chi *et al.* 2010a; Chi *et al.* 2010b; Chi *et al.* 2011; Chi *et al.* 2014) because of their ability to attach to ribosome and block protein synthesis during bacterial growth inhibition (Dobšíková *et al.* 2013). Furthermore, since the effects of antibiotics on enzymes activities were analysed *in vivo*, variations might be caused by the additive toxicity of the parent compounds and their metabolites on fish species (Lundén *et al.* 2002). However, further studies are required to gain more insights into these assumptions.

The results on growth performance are further complicated by a few antibiotics, which promoted growth in different fish species fed on various dietary doses of therapeutic (Johnson & Smith 1994; Wise & Johnson 1998; He *et al.* 2011a; Reda *et al.* 2013; Zhou *et al.* 2018a), prophylactic (Sanchez-Martínez *et al.* 2008) and different doses (Gaunt *et al.* 2003), coupled with environmental relevant and toxicological bath exposure (Charvatova *et al.* 2015), in dose-dependent manners (Gaunt *et al.* 2003; Charvatova *et al.* 2015). The authors attributed the improved growth performance to similar palatability of feeds, feed consumption and reduced feed conversion ratio (Johnson & Smith 1994; Gaunt *et al.* 2003; Reda *et al.* 2013). However, dietary OTC and FFC improved growth in *I. punctatus* but feed intake, feed conversion efficiency and palatability were not affected (Gaunt *et al.* 2003; Sanchez-Martínez *et al.* 2008), suggesting other reasons maybe responsible for enhanced growth in fish treated with antibiotics. Accordingly, increased growth has been related to decreased gut microbiota, leading to increased nutrient utilization and reduced host maintenance costs (Gaskins *et al.* 2002; Dibner & Richards 2005; Sanchez-Martínez *et al.* 2008) and reduced gut wall and villus lamina propria (Jukes *et al.* 1956), which enhance nutrient absorption (Limbu *et al.* 2018; Limbu *et al.* 2019). Evidently, *O. niloticus* ♀ × *O. aureus* ♂


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**Table 2** The effects of antibiotics administered through medicated feeds or bath on growth performance, feed utilization, survival and body development in cultured fish

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| Oxytetracycline | Dietary; 8.4, 16.8 and 33.5 mg $kg^{-1}$ diet; 28 and 56 days | *Ictalurus punctatus*; 8.7 g and 20.3 g | No differences in growth and feed efficiency | Rawles *et al*. (1997) |
| Oxytetracycline | Dietary; 82.5, 248 and 413 mg $kg^{-1}$ $day^{-1}$; 10 days | *Perca flavescens;* 40 to 95 g | No difference in fish mass and survival rate between treated and control.<br>Feed consumption ranged from 50% to 100% | Gaikowski *et al*. (2003b) |
| | Dietary; 82.5, 248 and 413 mg $kg^{-1}$ $day^{-1}$; 10 days | ‡*Morone saxatilis* × *M. chrysops*; 90 to 180 g | Decreased growth in treated than controls<br>Feed consumption and survival rate were not affected | |
| | Dietary; 82.5, 248 and 413 mg $kg^{-1}$ $day^{-1}$; 20 days | *Sander vitreus*; 100 to 150 g | Similar fish mass between treated and control fish<br>Reduced feed consumption in treated than control fish | |
| Oxytetracycline | Dietary; 50 mg $kg^{-1}$ dose; 77 days | *Ictalurus punctatus*; 10.16 ± 0.23 g | Mean weight, length and condition factor were enhanced in treated than control fish<br>No differences in feed intake, survival rate and feed conversion index | Sanchez-Martínez *et al*. (2008) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight; 50 days | *Cyprinus carpio*; 185.5 ± 35.1 g | No differences in total weight length, survival rate and condition factor between treated and control fish<br>Normal feeding behaviour | Dobšíková *et al*. (2013) |
| Oxytetracycline | Dietary; 82.8 mg $kg^{-1}$ body weight; 10 days<br>Dietary; 82.8 mg $kg^{-1}$ body weight; 10 days<br>Dietary; 82.8 mg $kg^{-1}$ body weight; 10 days | *Ictalurus punctatus*; 8.44 ± 2.05 g<br>*Oreochromis niloticus*; 235.61 ± 150.13 g<br>‡*Morone saxatilis* ♂ × *M. chrysops* ♀; 281.23 ± 65.80 g | No differences in survival rate | Topic Popovic *et al*. (2012) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ diet; 84 days | *Oreochromis niloticus*; 20 ± 0.09 g | Increase in final body weight, weight gain and decrease in food conversion for treated than the control fish<br>No differences in survival rate | Reda *et al*. (2013) |
| Oxytetracycline | Dietary; 10 mg $pound^{-1}$ of feed; 43 days | *Oncorhynchus mykis*; ~20.83 to ~ 33.33* g | No growth stimulation for treated than control fish | Wagner (1954) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 14 days | *Oncorhynchus kisutch*; 273 g | No differences in growth and survival rate | Nakano *et al*. (2018) |
| Oxytetracycline | Dietary; 1000 mg $kg^{-1}$ feed; 6 h | *Oncorhynchus mykiss*; 628 ± 151 g | No differences in growth.<br>Reduced feed intake for treated than control fish<br>Higher survival rate in treated than control fish | Hustvedt *et al*. (1991) |
| Oxytetracycline | Exposure; 0.005, 0.050, 0.500, 5.000 and 50.00 mg $L^{-1}$; 96 h | *Oncorhynchus mykiss*; 9.24 ± 0.23 g | No differences in survival rate | Rodrigues *et al*. (2017a) |


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**Table 2** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| Oxytetracycline | Dietary; 3 g $kg^{-1}$ diet; 21 days | *Salmo salar*; 172 g | No effects of treatment on final body weight, length and survival rate | Refstie *et al*. (2006) |
| Oxytetracycline | Exposure; 75, 100, 150, 300, 600 and 900 mg $L^{-1}$; 96 h | *Danio rerio*; eggs and embryo | Treated fish had delayed hatching of embryos | Oliveira *et al*. (2013) |
| Oxytetracycline | Dietary; 20 g $kg^{-1}$ diet; 65 days | *Salmo salar*; 170 g | Treated fish had spinal deformities and low feed intake than control | Toften and Jobling (1996) |
| | | *Salvelinus alpinus*; 287 g | No deformities and no significant differences in feed intake | |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ diet; 42 days | *Danio rerio;* 0.1 g | No differences in growth between treated and control fish | Zhou *et al*. (2018a) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$; 42 days | *Danio rerio;* 0.1 g | No effect on growth between treated and control fish | Zhou *et al*. (2018b) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | No differences in growth between treated and control fish | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Lower weight gain for treated than control fish | Limbu *et al*. (2018) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 65 days | *Oreochromis niloticus;* 8.45 ± 0.15 g | Lower weight gain, feed efficiency and protein content for treated than control fish | Limbu *et al*. (2019) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 35 days | *Oreochromis niloticus;* 9.50 ± 0.08 g | Lower weight gain, feed efficiency, protein efficiency ratio and survival rate for treated than control fish | Limbu *et al*. (2020) |
| Chlortetracycline | Dietary; 10 mg $pound^{-1}$ of feed; 43 days | *Oncorhynchus mykiss*; ~20.83 to ~ 33.33* g | No growth stimulation for treated than control fish | Wagner (1954) |
| Tetracycline | Exposure; 0.002, 0.01, 0.02, 0.2, 2000 and 20 mg $L^{-1}$; 96 h | *Danio rerio*; embryos after 4 h postfertilization | Decreased growth and survival rate, delayed hatching, increased yolk sac area and lack of swim bladder in treated than control fish | Zhang *et al*. (2015) |
| Tetracycline | Exposure; 0.01, 1, 100 μg $L^{-1}$; 120 h | *Danio rerio;* larvae | No differences in survival rate. Reduced body length in treated fish | Qiu *et al*. (2020) |
| Tetracycline | Exposure; 90, 180, 270, 360, 450, 540, 630, 720, 810 and 900 μg $L^{-1}$; 96 h | *Cyprinus carpio;* Oocytes | Treated fish had malformation in tail, modified chorda structure, pericardical oedema, scoliosis and malformations of the heart. | Escobar-Huerfano *et al*. (2020) |
| Florfenicol | Dietary; 5 mg $kg^{-1}$ body weight; 84 days | *Oreochromis niloticus*; 20 ± 0.09 g | Increase in final body weight and weight gain and decrease in food conversion for treated than the control fish | Reda *et al*. (2013) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Oreochromis niloticus*; ~500 g | Decreased growth in treated than control fish<br>No effect on feed consumption and survival rate | Gaikowski *et al*. (2015) |
| | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; ~500 g | Reduced growth in treated than control fish<br>No differences in feed consumption and survival rate | |


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**Table 2** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| Florfenicol | Dietary; 15, 45 and 75 mg $kg^{-1}$ body weight $day^{-1}$; 20 days | *Oreochromis* sp.; 45.8 ± 10.5 g | Reductions in final weight, mean weight, body size and feed consumption in treated relative to control fish<br>No differences in survival rate | Gaikowski *et al*. (2013) |
| Florfenicol | Dietary; 5, 10 and 20 mg $kg^{-1}$ body weight $day^{-1}$; 11 days | *Salmo salar*; 11.5 g | No difference in mean weight and feed palatability<br>Higher survival rate in treated than control fish | Inglis *et al*. (1991) |
| Florfenicol | Dietary; 15, 45 and 75 mg $kg^{-1}$ body weight $day^{-1}$; 20 days | ‡*Morone chrysops* × *M. saxatilis*; 13.6 ± 1.6 g | Comparable mean weight length and survival rate between treated and control fish | Straus *et al*. (2012) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 112 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 46.88 ± 0.38 g | Improved growth performance in treated than the control fish<br>No differences in survival rate | He *et al*. (2011a) |
| Florfenicol | Dietary; 10, 30 and 50 mg $kg^{-1}$ body weight $day^{-1}$; 20 days | *Ictalurus punctatus*; 19.1 to 22.3 g | No effect on growth and survival rate<br>Treated fish had a reduced feed intake | Gaikowski *et al*. (2003a) |
| Florfenicol | Dietary; 10, 20, 40 and 100 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Ictalurus punctatus*; 5-month-old | Increased weight gain and survival in treated than control fish<br>No difference in feed palatability | Gaunt *et al*. (2003) |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Gadus morhua*; 1377 ± 329 g | Lower feed consumption in treated than control fish | Melingen and Samuelsen (2011) |
| Florfenicol | Dietary; 15, 45, or 75 mg $kg^{-1}$ body weight $day^{-1}$; 20 days | *Oreochromis* sp.; 45.8 ± 10.5 g | Increased body weight gain of the control compared to treated fish | Di Salvo *et al*. (2013) |
| Chloramphenicol | Dietary; 10 mg $pound^{-1}$ of feed; 43 days | *Oncorhynchus mykis*; ~20.83 to ~ 33.33* g | No growth stimulation for treated and control fish | Wagner (1954) |
| Chloramphenicol | Exposure; 2.5, 5.0 and 10.0 mg $L^{-1}$; 15 days | *Clarias gariepinus*; 16.42 ± 1.74 g | Treated fish had loss of equilibrium, erratic swimming, circling movement and hyperactivity<br>No differences in survival rate | Nwani *et al*. (2014) |
| Chloramphenicol | Exposure; 0.03, 0.06, 0.12, 0.24 and 0.48 mM; | *Danio rerio*; Embryos, 2 h postfertilization and 2 h posthatching larvae | Delayed hatching, pericardial oedema and hemagglutination in treated than control larvae<br>Reduced survival in treated than control larvae | Song *et al*. (2010) |
| Kanamycin | Exposure; 0.36, 0.72, 1.44, 2.88 and 5.76 mM; | *Danio rerio*; Embryos, 2 h postfertilization and 2 h posthatching larvae | Delayed hatching and axial malformation in treated than control larvae<br>Reduced survival in treated than control embryos and larvae | |
| Enrofloxacin | Exposure; 0.01, 1, 100 μg $L^{-1}$; 120 h | *Danio rerio;* larvae | No differences in survival rate. Reduced body length in treated fish | Qiu *et al*. (2020) |
| Enrofloxacin | Exposure; 0.1, 0.8, 10 and 100 mg $L^{-1}$; 14 days | *Oreochromis niloticus*; 11 ± 2 g | Treated fish has short time impaired swimming behaviour with no or very slow movements | Andrieu *et al*. (2015) |
| Ciprofloxacin | Exposure; 0.05, 0.4, 5.0 and 50 mg $L^{-1}$; 14 days | *Oreochromis niloticus*; 11 ± 2 g | No effect on swimming behaviour between treated and control fish | |




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**Table 2** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| Ciprofloxacin | Exposure; 0.0007, 0.1, 0.65, 1.1 and 3.0 mg $L^{-1}$; 28 days | *Danio rerio*; 9 ± 0.8 g | No difference in specific growth rate between treated and control fish<br>No pathological changes in organs of exposed fish | Plhalova *et al*. (2014) |
| Fluoroquinolone | Dietary; 10000 mg $kg^{-1}$; 10 days | *Oncorhynchus mykiss*; Not given | Reduced specific growth rate and voluntary feed intake in treated than control fish<br>No differences in survival rate | Boujard *et al*. (1997) |
| Quinocetone | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 2.67 ± 0.01 g | No differences in growth performance, feed utilization and survival rate between treated and control fish | Zhou *et al*. (2009) |
| Norfloxacin | Exposure; 0.1, 1, 10 and 30 mg $L^{-1}$; 28 days | *Danio rerio*; 30 days old | No differences in specific growth and survival rate between treated and control fish | Bartoskova et al. (2013) |
| Norfloxacin | Exposure; 0.0001, 0.1, 1.0, 5.0 and 10.0 mg $L^{-1}$; 34 days | *Cyprinus carpio*; Eggs and embryos twenty-four hours after fertilization | Increased total body length in exposed than control fish<br>No differences in survival rate | Charvatova *et al*. (2015) |
| Norfloxacin | Exposure; 2, 20 and 200 μg $L^{-1}$; 150 days | *Danio rerio;* larvae to adult | No difference on body weight and condition factor | Yan *et al*. (2016) |
| Clinafloxacin | Exposure; 10 mg L; 7 days | *Pimephales promelas*; larvae more than 24 h old | Reduced survival rate in treated than control fish | Robinson *et al*. (2005) |
| Oxolinic acid | Dietary; 10000 mg $kg^{-1}$; 6-h period | *Oncorhynchus mykiss*; 628 + 151 g | No effect on growth and feed intake between treated and control fish | Hustvedt *et al*. (1991) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ diet; 42 days | *Danio rerio;* 0.1 g | Increased weight gain of treated fish | Zhou *et al*. (2018a) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$; 42 days | *Danio rerio;* 0.1 g | No effect on growth between treated and control fish | Zhou *et al*. (2018b) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Lower weight gain for treated than control fish | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ diet; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Lower weight gain for treated than control fish | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 0.1, 1, 10, 100 μg $L^{-1}$; 120 h | *Danio rerio;* embryos | Delayed hatching of treated embryos. Reduced body length of treated larave | Liu *et al*. (2020b) |
| Sulfamethoxazole | Exposure; 2, 20 and 200 μg $L^{-1}$; 150 days | *Danio rerio;* larvae to adult | Reduced body weight and condition factor of treated than control fish | Yan *et al*. (2016) |
| Sulfadimethoxine/ormetoprim | Dietary; 350/70, 690/140 and 1390/280 mg $kg^{-1}$; 28 and 56 days | *Ictalurus punctatus*; 8.7 g and 20.3 g | No differences in growth and feed efficiency after 56 days<br>Higher survival rate for treated than control fish | Rawles *et al*. (1997) |
| Sulfadimethoxine/ormetoprim | Dietary; 40.5 mg $kg^{-1}$ diet; 28 days | *Ictalurus punctatus*; 4.55 ± 0.07 g | Higher weight gain and survival rate for treated than control fish | Wise and Johnson (1998) |
| Sulfadimethoxine/ ormetoprim | Dietary; 50 mg $kg^{-1}$ body weight; 77 days | *Ictalurus punctatus*; Not provided | Suppressed growth in treated than control group<br>No reduction in feeding appetite and condition factor | Rábago-Castro *et al*. (2006) |


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**Table 2** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| Sulfadimethoxine/ormetoprim | Dietary; 1.65% Romet-30; 7 days | *Ictalurus punctatus*; 10–12 g | Decreased growth and feed palatability in treated than control fish | Robinson *et al*. (1990) |
| Sulfadimethoxine/ormetoprim | Dietary; 50 mg $kg^{-1}$ body weight $day^{-1}$; 5 days | *Ictalurus punctatus*; 7–10 cm | Increased weight and survival rate for treated than control fish | Johnson and Smith (1994) |
| Sulfamonomethoxine | Exposure; 0.01, 1, 100 μg $L^{-1}$; 120 h | *Danio rerio;* larvae | No differences in survival rate. Reduced body length in treated fish | Qiu *et al*. (2020) |
| Sulfamerazine | Dietary; 200 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | *Salmo trutta*; ~500 g | Reduction in growth rate for treated than control fish | Snieszko and Wood (1955) |
| | | *Salmo gairdneri*; ~500 g | No effect in growth between treated and control fish | |
| | | *Salvelinus fontinalis*; ~500 g | Reduction in growth, survival rate and loss of appetite in the treated than control fish | |
| Sulfisoxazole | Dietary; 200 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | *Salmo trutta*; ~500 g<br>*Salmo gairdneri*; ~500 g<br>*Salvelinus fontinalis*; ~500 g | No effect in growth rate for all tested species | |
| Salinomycin | Dietary; 15, 30 and 60 mg $kg^{-1}$ body weight $day^{-1}$; 8 days | *Ictalurus punctatus*; Not given | Reduced body weight gain in treated than control fish<br>Poor feed conversion ratio and palatability for treated than control fish<br>No differences in survival rate | Manning *et al*. (2013) |
| Sarafloxacin | Dietary; 2, 6, 10, and 14 mg $kg^{-1}$; 5 and 10 days | *Ictalurus punctatus*; 6.66 g | Higher survival rate in treated than control fish | Thune and Johnson (1992) |
| Amoxicillin | Exposure; 75, 128, 221, 380, 654 and 1125 mg $L^{-1}$; 96h | *Danio rerio*; Eggs | Treated eggs had premature hatching | Oliveira *et al*. (2013) |
| Penicillin | Dietary; 10 mg $pound^{-1}$ of feed; 43 days | *Oncorhynchus mykiss*; 1.25 to 2 inches | No growth stimulation for treated than control fish | Wagner (1954) |
| Flavomycin | Dietary; 8 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 2.67 ± 0.01 g | No differences in growth performance, feed utilization and survival rate between treated and control fish | Zhou *et al*. (2009) |
| Cefalexin | Exposure; 1.3, 2.5, 5 and 10 mg $L^{-1}$; 96 h | *Pomatoschistus microps*; ≈ 2.5 cm total length | No differences in total length and weight between treated and control fish<br>Decreased predatory performance of treated than control fish | Fonte *et al*. (2016) |
| Gentamycin | Exposure; 10 mg $mL^{-1}$; 8 to 12 h | *Danio rerio*; larvae 50 to 55 h postfertilization | Treated fish has acute renal failure | Hentschel *et al*. (2005) |
| Streptomycin | Exposure; 0.1, 1.0, 10.0 μg $mL^{-1}$; 10 days | *Danio rerio*; larvae | Increased mortality among treated fish | Pindling *et al*. (2018) |
| Cefotaxime sodium | Exposure; 0.01, 1, 100 μg $L^{-1}$; 120 h | *Danio rerio;* larvae | No differences in survival rate<br>Reduced body length in treated than control fish | Qiu *et al*. (2020) |
| Cefotaxime | Exposure; 3 - 4 mL; 20 min | *Danio rerio;* larvae | Decreased swimming time in exposed than control fish | Han *et al*. (2018) |


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**Table 2** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect | Reference(s) |
|---|---|---|---|---|
| †β-diketone antibiotics | Exposure; 6.25, 12.5, 25 and 50 mg $L^{-1}$; 90 days | *Danio rerio;* 90 days postfertilization | Shedding of gill filamentous epithelial cells and deformation or disappearance of red blood cells | Li *et al*. (2016) |
| †β-diketone antibiotics | Exposure; 4.69, 9.38, 18.75 and 37.5 mg $L^{-1}$; 90 to 120 days postfertilization | *Danio rerio*; larvae 30 days postfertilization | Reduction in heart rate and stimulated spontaneous movement in treated fish | Yin *et al*. (2014) |
| †β-diketone antibiotics | Exposure; 2.34, 9.38 and 37.5 mg $L^{-1}$; 96 to 120 h postfertilization | *Danio rerio*; embryos from 6 to 96 h posthatching | Higher swimming speed, delayed hatching, curved body axis, pericardial oedema, uninflated swim bladder and yolk sac oedema for treated fish<br>Reduced heart rates for treated fish embryos than control fish | Wang *et al*. (2014) |
| †β-diketone antibiotics | Exposure; 9.38, 18.75, 37.5, 75 and 150 mg $L^{-1}$; 6 to 72 h postfertilization<br>4.69, 9.38 and 18.75; 6 to 72 h postfertilization | *Danio rerio*; Embryos 72 and 120 h postfertilization | Decreased hatching, survival and heart beats rates for treated than control fish<br>Higher and basal swimming speed for exposed than control fish | Zhang *et al*. (2016) |
| †β-diketone antibiotics | 30, 60 and90 mg $L^{-1}$; 60 days | *Danio rerio*; 2 months old | Mitochondria swelling, mitochondrial cristae reduction, broken cristae with disordered arrangement and vacuous matrix for treated fish<br>Altered genes expressions for heart development and skeletal muscle formation such as the actin alpha 1 (*acta1a*), myosin light chain 7 (*myl7*) and *gle1b* in treated fish | Zhang *et al*. (2016) |

β-diketone antibiotics (DKAs) are a mixture of four fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin and enrofloxacin) and two tetracyclines (chlortetracycline and doxycycline). †β-diketone was counted as an individual antibiotic. ‡Hybrids were counted as individual species. *The weight was obtained after converting inches to grams by using the factor 1 g = 0.06 cubic inches.

hybrids treated with FFC improved growth performance, wherein intestinal bacterial count and diversity were decreased (He *et al*. 2011a). Surprisingly, flavomycin (FVC) and quinocetone (QCT) did not affect growth performance in *O. niloticus* ♀ × *O. aureus* ♂ hybrids, but intestinal bacterial abundance and diversity were decreased (Zhou *et al*. 2009). In addition, *O. niloticus* treated with OTC and SMZ, both as dietary and bath, had reduced muscularis thickness and villi width (Limbu *et al*. 2018; Limbu *et al*. 2019) and villi height and goblet cells (Limbu *et al*. 2019) as attempts to increase nutrients absorption. However, in these studies, the growth performance was reduced only in fish fed on dietary OTC (Limbu *et al*. 2019) and both dietary and bath SMZ (Limbu *et al*. 2018), but not dietary OTC (Limbu *et al*. 2018). These results emphasize specific effects of antibiotics on improved growth performance depending on types of antibiotics, their doses and exposure methods and time.

In summary, the effects of antibiotics in fish growth are still inconsistent depending on dose and time (Rawles *et al*. 1997; Sanchez-Martínez *et al*. 2008; Gaikowski *et al*. 2013; Yan *et al*. 2016), fish species (Snieszko & Wood 1955; Gaikowski *et al*. 2003b) and their size (Rawles *et al*. 1997; Wise & Johnson 1998) and exposure method (Limbu *et al*. 2018; Limbu *et al*. 2019) (Table 2). The interspecific variation in sensitivity to antibiotics can also be related to the mechanism of toxicity of the parent compounds and their metabolites and the capacity to adapt and minimize the effects, developed by each fish species. These variations are related to bioavailability of parent antibiotics and their metabolites in the water used to culture fish, which may be modulated by abiotic factors such as pH, temperature and


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light. Therefore, results obtained in a particular fish species subjected to a specific antibiotic should not be generalized to other species without experimental support. Future studies should focus on influence of diet composition (Limbu *et al.* 2019; Limbu *et al.* 2020), nutritional sources and fish history background prior to antibiotics treatments in order to explain the contradicting results obtained in fish growth. In addition, the influence of water quality parameters in the culture media for fish on antibiotics toxicity should also be elucidated (Weinrauch *et al.* 2020). Moreover, the additive effect of antibiotics used in aquaculture and those existing in the water used for fish culture, such as river water or seawater, should be studied. In fact, Limbu *et al.* (2018) found appreciable levels of antibiotics in the water used to culture fish in the control treatment, indicating that the culture water contained some levels of the studied antibiotics.

## Survival rate

Similar to growth performance, survival rate determines the yield and economic production of aquaculture. Numerous studies, published over the years, assessed the effects of antibiotics on survival rate of cultured fish yielding results in a similar pattern to growth performance (Table 2). None interference of antibiotics on survival rate was obtained in several fish species using various doses of dietary (Boujard *et al.* 1997; Gaikowski *et al.* 2003a; Gaikowski *et al.* 2003b; Refstie *et al.* 2006; Sanchez-Martínez *et al.* 2008; Zhou *et al.* 2009; He *et al.* 2011a; Straus *et al.* 2012; Topic Popovic *et al.* 2012; Dobšíková *et al.* 2013; Gaikowski *et al.* 2013; Manning *et al.* 2013; Gaikowski *et al.* 2015; Limbu *et al.* 2018; Nakano *et al.* 2018) and bath treatments (Bartoskova *et al.*, 2013; Charvatova *et al.* 2015; Rodrigues *et al.* 2017a; Limbu *et al.* 2018; Qiu *et al.* 2020; Liu *et al.* 2020b).

However, various doses of dietary (Snieszko & Wood 1955; Limbu *et al.* 2018; Limbu *et al.* 2020) and bath exposure using several antibiotics (Robinson *et al.* 2005; Song *et al.* 2010; Zhang *et al.* 2015; Zhang *et al.* 2016) reduced survival rate of various treated fish due to the toxicity of the antibiotics (Robinson *et al.* 2005; Song *et al.* 2010; Zhang *et al.* 2015). Reduced survival rate discourages fish farmers because it minimizes yield and economic gains from aquaculture production (Shoko *et al.* 2019). The aquaculture production is further worsened by scientific evidence indicating that, antibiotics depress egg production of parental fish after exposure to single or combined environmental relevant concentrations for long-term (Yan *et al.* 2016), which is a realistic exposure situation. This may reduce aquaculture fingerlings production required to perpetuate the industry. On the other hand, various doses of dietary antibiotics (Hustvedt *et al.* 1991; Inglis *et al.* 1991; Thune & Johnson 1992; Johnson & Smith 1994; Rawles *et al.* 1997; Wise & Johnson 1998; Jarau *et al.* 2019) increased the survival rate of treated fish due to effective killing or inhibition of pathogenic bacteria (Inglis *et al.* 1991; Johnson & Smith 1994; Jarau *et al.* 2019). In general, distinct antibiotics induce controversial effects on fish survival rate based on exposure methods (Hustvedt *et al.* 1991; Rodrigues *et al.* 2018c) and doses and time (Topic Popovic *et al.* 2012; Reda *et al.* 2013; Limbu *et al.* 2018) as observed on growth performance (Table 2). The variations in the results might also be caused by existence of antibiotics in the water used to culture fish and those administered during the experiments (Limbu *et al.* 2018).

## Body malformation and damage

Valuable scientific clues exist on the ability of antibiotics to induce body malformation and damage in cultured fish (Table 2). Discernibly, the exposure of cultured fish to bath antibiotics at toxicological concentrations induced body malformations in a dose-dependent (Oliveira *et al.* 2013; Andrieu *et al.* 2015; Zhang *et al.* 2015; Zhang *et al.* 2016; Almeida *et al.* 2019) and interactive manners (Song *et al.* 2010; Wang *et al.* 2014; Yin *et al.* 2014) due to their toxicity. Similarly, body damages were also evident after bath exposure at environmental relevant levels (Almeida *et al.* 2019; Escobar-Huerfano *et al.* 2020; Liu *et al.* 2020b), sublethal concentrations (Nwani *et al.* 2014; Li *et al.* 2016) and excessive dietary dose (Toften & Jobling 1996). In these studies, treated fish had delayed and premature hatching (Zhang *et al.* 2015; Liu *et al.* 2020b), higher swimming speed (Yin *et al.* 2014; Zhang *et al.* 2016), impaired swimming behaviour (Andrieu *et al.* 2015), reduced swimming time (Han *et al.* 2018) and spinal fractures (Toften & Jobling 1996). Others had malformation in tail, modified chorda structure, pericardial oedema, scoliosis and malformations of the heart (Li *et al.* 2016; Escobar-Huerfano *et al.* 2020) and reduced heart rate beats (Wang *et al.* 2014; Yin *et al.* 2014; Zhang *et al.* 2016). Furthermore, the fish treated with antibiotics had mitochondria swelling, mitochondrial cristae reduction, broken cristae with disordered arrangement and vacuous matrix, in addition to altered genes expressions for heart development and skeletal muscle formation (Zhang *et al.* 2016). Increasing evidences also show that, bath treatment induces severe toxic body effects than oral dietary, at similar doses (Andrieu *et al.* 2015), due to higher bioavailability, easier digestibility and assimilation rates of the former exposure method than the



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latter (Limbu *et al.* 2018). Nevertheless, antibiotics induced species-specific malformation (Toften & Jobling 1996) and similar effects at different doses and time (Wang *et al.* 2014; Yin *et al.* 2014; Zhang *et al.* 2016), indicating that, both exposure to acute lower and chronic higher doses are toxic to fish (Table 2).

In a nutshell, antibiotics induce several body malformation and damage, which cause physiological and metabolic dysfunctions. However, some antibiotics maybe beneficial in different growth stages of animals. For instance, CAP and trimethoprim–sulfamethoxazole (TMP-SMX) increased hatching rate of eggs compared to larvae (Feuerbacher *et al.* 2017), while penicillin and streptomycin reduced the prevalence of *Flavobacterium psychrophilum* on the surface and inside of eggs (Oplinger *et al.* 2015), and gentamicin prevented effectively the initial postsurgical infection in hybrid striped bass *Morone saxatilis* × *M. chrysops* antennae (Isely *et al.* 2002), due to their ability to kill or inhibit pathogenic bacteria proliferation (Inglis *et al.* 1991; Johnson & Smith 1994; Jarau *et al.* 2019). Future studies linking the observed body malformation to nutrients, physiology and metabolism in cultured fish are needed. Nevertheless, it is imperative to understand the effects of antibiotics on microbiota abundance and diversity in cultured fish species.

## Microbiota dysbiosis

Intestinal microbiota are critical to host nutritional, physiological, pathological and metabolic activities such as digestion, nutrition, biotransformation, innate immunity, disease resistance, proliferation of epithelial cells and functional maturation of the gut (Navarrete *et al.* 2009; Situmorang *et al.* 2014; Giatsis *et al.* 2015; Liu *et al.* 2016). Antibiotics treatment in cultured fish do not target only pathogenic bacteria but also affect other symbiotic microbiota (Done *et al.* 2015). Vast experimental evidences from feeding and exposure trials in cultured fish, suggest antibiotics affect microbiota (Table 3). Certainly, antibiotics decreased the abundance and diversity of intestinal microbiota in various fish species regardless of exposure methods, doses and time, after dietary (Austin & Al-Zahrani 1988; Navarrete *et al.* 2008; Zhou *et al.* 2009; He *et al.* 2010; He *et al.* 2011a; He *et al.* 2012; Limbu *et al.* 2018; Zhou *et al.* 2018a; Abdelhamed *et al.* 2019; Limbu *et al.* 2019) and bath exposure to environmental relevant (Limbu *et al.* 2018; Zhou *et al.* 2018b; Jia *et al.* 2020) and toxicological concentrations (Almeida *et al.* 2019) (Table 3), due to their ability to inhibit normal/sensitive/susceptible bacteria (Navarrete *et al.* 2008; Romero *et al.* 2012; Zhou *et al.* 2018b). These results suggest adverse influence to the host fish metabolism because most phyla reduced are required for normal functions of the intestine such as Actinobacteria including antibiotics producing *Streptomyces* species (Zhou *et al.* 2009; He *et al.* 2010; He *et al.* 2011a; He *et al.* 2012; Limbu *et al.* 2018), Firmicutes such as Lachnospiraceae family (Zhou *et al.* 2009; Limbu *et al.* 2018; Abdelhamed *et al.* 2019), Bacteroidetes including *Cloacibacterium* species involved in carbohydrate metabolism (Limbu *et al.* 2018; Zhou *et al.* 2018b; Abdelhamed *et al.* 2019), Proteobacteria such as symbiotic *Rhizobiales* species (Zhou *et al.* 2009; Limbu *et al.* 2018; Zhou *et al.* 2018b) and Cyanobacteria (He *et al.* 2010; Limbu *et al.* 2018). Therefore, the reduction in these phyla suggests adverse host microbiota dysbiosis, because they are mutually related to beneficial intestinal health (Desai *et al.* 2012).

The reduction in the normal bacteria in cultured fish intestines by antibiotics facilitates the proliferation of pathogenic, opportunistic and resistant bacteria species due to reduced competition for space and nutrients (Navarrete *et al.* 2008) (Fig. 1). Evidently, antibiotics increased bacteria, which are pathogenic such as *Aeromonas* species (Navarrete *et al.* 2008; Zhou *et al.* 2018a), *Poryphyromonads* species (Limbu *et al.* 2019), *Enterococcus* species (Zhou *et al.* 2018a) and *Microbacterium* species (Limbu *et al.* 2019; Limbu *et al.* 2020), opportunistic including *Escherichia* species (Abdelhamed *et al.* 2019) and *Sphingomonas* species (Pindling *et al.* 2018) as well as the proportion of tetracycline resistance bacteria (Jia *et al.* 2020) in various fish intestines. Moreover, some of the increased bacteria species developed antibiotics resistance genes such as *tetE* and *tetD/H* (Austin & Al-Zahrani 1988), *tetE* (Navarrete *et al.* 2008) and *tetA*, *tetC*, *tetG, tetK* and *tetZ* (Jia *et al.* 2020) and class 1 integrons, responsible for the horizontal transfer of resistance genes (Pindling *et al.* 2018). Antibiotics cause resistance genes development in cultured fish through their modification by bacterial enzymes such as phosphotransferases (Vieira & Vieira 1997) and production of excessive reactive oxygen species (ROS) (Wang *et al.* 2013). The excessive ROS generated cause mutant strains that are sensitive to the applied antibiotic but resistant to other antibiotics (Kohanski *et al.* 2010).

Generally, antibiotics eradicate the normal intestinal microbiota of farmed fish and facilitate the proliferation of pathogenic, opportunistic and resistant bacteria colonization (Fig. 1), suggesting impaired host nutritional, physiological, pathological and metabolic functions. However, studies relating these host functions and toxicity of antibiotics to the observed microbiota dysbiosis in fish are currently limited. Ideally, these can be discovered by studying the effects of antibiotics in host fish by using gnotobiotic models (Zhang *et al.* 2020), while integrating advances in biochemical, molecular, omics and next-generation


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**Table 3** The effects of antibiotics treatments on microbiota in cultured fish

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect on microbiota | Reference(s) |
|---|---|---|---|---|
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ diet; 10 days | *Salmo salar*; 14.4 ± 4.7 g | Reduced diversity of bacteria. Increase in pathogenic bacteria such as *Aeromonas* species (Proteobacteria) and resistant genes such as *tetE* | Navarrete *et al*. (2008) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ diet; 42 days | *Danio rerio;* 0.1 g | Decreased intestinal microbial richness<br>Increased Proteobacteria such as *Aeromonas* and Firmicutes such as *Enterococcus* species | Zhou *et al*. (2018a) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$; 42 days | *Danio rerio;* 0.1 g | Decreased Proteobacteria, Bacteroidetes and Planctomycetes phyla | Zhou *et al*. (2018b) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Eradicated species in Bacteroidetes and Firmicutes phyla | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Eradicated useful species such as *Cloacibacterium* (Bacteroidetes) and Lachnospiraceae family (Firmicutes) | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 0.1, 10, 10000 μg $L^{-1}$; 60 days | *Denio rerio*: four months old | Decreased species richness and diversity at the highest concentration | Almeida *et al*. (2019) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 65 days | *Oreochromis niloticus*; 8.45 ± 0.15 g | Increased pathogenic species such as *Poryphyromonads* (Bacteroidetes) and *Microbacterium* (Actinobacteria) | Limbu *et al*. (2019) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ diet; 35 days | *Oreochromis niloticus*; 9.50 ± 0.08 g | Increased Actinobacteria pathogenic bacteria such as *Microbacterium* species | Limbu *et al*. (2020) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ diet; 10 days | *Salmo gairdneri*: 15 g | Increased bacterial numbers | Austin and Al-Zahrani (1988) |
| Tetracycline | Exposure: 0.285 and 2.85 μg $L^{-1}$; 7 and 21 days | *Carassius auratus*; (12.00 ± 1.78 g) | Increased proportion of tetracycline resistance bacteria and genes | Jia *et al*. (2020) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 50.89 ± 0.27 g | Reduced the number of intestinal bacteria.<br>Eradicated useful Actinobacteria such as *Streptomyces* species | He *et al*. (2010) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 112 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 46.88 ± 0.38 g | Decreased bacteria count and diversity | He *et al*. (2011a) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 33.6 ± 0.20 g | Decreased total intestinal bacterial counts.<br>Eradicated Actinobacteria such as *Streptomyces* species | He *et al*. (2012) |
| Florfenicol | Dietary; 10 to 15 mg $kg^{-1}$ diet; 10 days | *Ictalurus punctatus*; 320 ± 39.61 g | Decreased Firmicutes and Bacteroidetes phyla<br>Increased Proteobacteria such as *Escherichia* species | Abdelhamed *et al*. (2019) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Reduced diversity of Proteobacteria and Cyanobacteria phyla | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ diet; 84 days | *Oreochromis niloticus;* 27.73 ± 0.81 g | Reduced diversity of Proteobacteria and Cyanobacteria phyla | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ diet; 42 days | *Danio rerio;* 0.1 g | Increased Firmicutes such as *Clostridiaceae* and *Bacillaceae* species | Zhou *et al*. (2018a) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$; 42 days | *Danio rerio;* 0.1 g | Reduced bacterial richness such as Proteobacteria, Bacteroidetes and Planctomycetes phyla | Zhou *et al*. (2018b) |
| Flavomycin | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 50.89 ± 0.27 g | Reduced one species of Fusobacteria phylum<br>Eradicated useful Actinobacteria such as *Streptomyces* species | He *et al*. (2010) |


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**Table 3** (continued)

| Antibiotic | Dosage, exposure method and duration | Species and size | Effect on microbiota | Reference(s) |
|---|---|---|---|---|
| Flavomycin | Dietary; 8 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 2.67 ± 0.01 g | Reduced Actinobacteria and Proteobacteria phyla | Zhou *et al.* (2009) |
| Quinocetone | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 56 days | ‡*Oreochromis niloticus* ♀ × *O. aureus* ♂; 2.67 ± 0.01 g | Reduced Actinobacteria, Firmicutes and Proteobacteria phyla | Zhou *et al.* (2009) |
| Erythromycin | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Salmo gairdneri*, 15 g | Reduced bacterial numbers | Austin and Al-Zahrani (1988) |
| Streptomycin | Exposure; 0.1, 1.0, 10.0 μg $mL^{-1}$; 10 days | *Danio rerio*; larvae | Increased abundance of resistant bacteria and class 1 integrons | Pindling *et al.* (2018) |
| Penicillin G | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Salmo gairdneri*; 15 g | Reduced bacterial numbers | Austin and Al-Zahrani (1988) |
| Oxolinic acid, | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Salmo gairdneri*; 15 g | Increased bacterial numbers | Austin and Al-Zahrani (1988) |
| Sulphafurazole | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Salmo gairdneri*; 15 g | Increased bacterial numbers | Austin and Al-Zahrani (1988) |

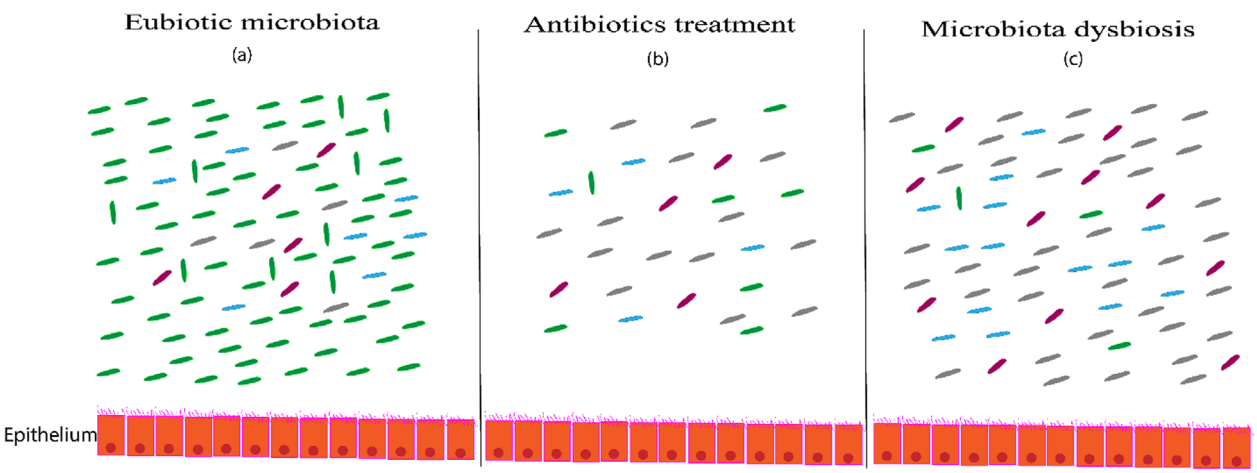



**Figure 1** The microbiota dysbiosis induced by antibiotics in global aquaculture production; (a) eubiotic microbiota dominated by normal bacteria before antibiotics application; (b) reduction in normal, some pathogenic and opportunistic bacteria during antibiotics administration; and (c) microbiota dysbiosis showing increased resistant, pathogenic and opportunistic bacteria after antibiotics application. Normal bacteria; Pathogenic bacteria; Opportunistic bacteria; Resistant bacteria.

sequencing (NGS) technologies. In addition, studies relating microbiota dysbiosis and short-chain fatty acids (SCFAs) production in fish, which are also affected by antibiotics (Romick-Rosendale *et al.* 2018), are required. The microbiota dysbiosis reported suggest modification of the beneficial host–microbiota relationship on immunity response and nutrients metabolism.

## Immunity and inflammatory responses

Antibiotics administered on fish affect several immune parameters (Li *et al.* 2010; Saravanan *et al.* 2012). The literature shows various effects of antibiotics on immunity response (Table 4) after exposure to single doses of dietary antibiotics (Grondel *et al.* 1987; Lundén *et al.* 1998; Lundén *et al.* 1999; Gaikowski *et al.* 2003a; Gaikowski *et al.* 2003b; Yonar *et al.* 2011; Guardiola *et al.* 2012; Yonar 2012; Limbu *et al.* 2018; Zhou *et al.* 2018a; Limbu *et al.* 2020) and bath exposure (Han *et al.* 2014; Li *et al.* 2016; Limbu *et al.* 2018; Zhou *et al.* 2018b; Liu *et al.* 2020b) and *in vitro* (Lundén *et al.* 2002), together with a combination of some antibiotics (Wise & Johnson 1998; Lundén & Bylund 2000) in different fish species. Similarly, several antibiotics compromised innate immunity response in various fish species, through promotion of inflammatory response (Barros-Becker *et al.* 2012; Limbu *et al.* 2018; Zhou *et al.* 2018b; Liu


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*et al*. 2020b), suppression of immunity genes (Guardiola *et al*. 2012; Limbu *et al*. 2020; Liu *et al*. 2020b) and antimicrobial peptides (Limbu *et al*. 2018). Reasonably, antibiotics exert immunosuppressive effects on T and B cells, neutrophils and macrophages (Lundén & Bylund 2000; Lundén *et al*. 2002; Han *et al*. 2014), due to their ability to penetrate into the cells where, either, they are retained or interact directly with the phagocytes, hence impairing cell functions due to their toxicity (Lundén *et al*. 2002; Li *et al*. 2016). Antibiotics may also affect immunity response through microbiota dysbiosis as indicated in the previous section. Reduced fish immunity indicates weakened defensive and detoxification mechanisms against viruses, bacteria and other opportunistic invaders. Accordingly, *D. rerio* pre-treated with SMZ and OTC diets (Zhou *et al*. 2018a) and baths (Zhou *et al*. 2018b) had higher mortality rate after *Aeromonas hydrophila* challenge, confirming the compromised immunity. Logically, the suppressed immunity was responsible for the reduced survival rate in fish treated with antibiotics reported previously in this review. This suggests devastating consequences of antibiotics administration on overall aquaculture production, because exposure of fish to lower antibiotics doses for shorter periods (Lundén & Bylund 2000) and longer periods (Lundén *et al*. 1998) produced similar immunity depressive effects as acute dietary exposure to higher doses (Yonar *et al*. 2011; Yonar 2012). Moreover, antibiotics induced immunity effects, which were sustained sometimes even after withdrawal period (Han *et al*. 2014). Therefore, antibiotics compromise immunity parameters in various cultured fish species regardless of the doses, exposure duration and routes. Nevertheless, β-diketone antibiotics induced immunotoxicity effects in *D. rerio* depending on dose and tissues sampled (Li *et al*. 2016).

Moreover, some antibiotics supplemented in dietary doses (Rijkers *et al*. 1980; Tafalla *et al*. 1999; Lundén & Bylund 2002; He *et al*. 2010; Guardiola *et al*. 2012; Reda *et al*. 2013), and injected into fish body (Rijkers *et al*. 1980) increased various immunological parameters due to the modulation of the immune system as an attempt to protect the fish body from the toxic effects of antibiotics (Tafalla *et al*. 1999; Li *et al*. 2016). This might be one of the reasons for the lack of antibiotics effects on immunity response after exposure to dietary (Tafalla *et al*. 1999; Lundén & Bylund 2002; He *et al*. 2011a) and bath (Li *et al*. 2016; López Nadal *et al*. 2018) treatments in various fish species. The fish body in these treatments perhaps improved their immune response and thus counteracted the immunosuppressive effects induced by antibiotics. Presumably, compromised immunity was one of the reasons for the reduced growth performance in the previous section of our review. Thereof, the fish in antibiotics treatments diverted more energy to counteract immunosuppressive effects than somatic growth. Collectively, exposures of fish to antibiotics in either diets, bath or injection suppress immune responses, which affect defensive and detoxification mechanisms. Challenging the fish treated with antibiotics by using pathogenic bacteria as done by Zhou *et al*. (2018a), Zhou *et al*. (2018b) and Hernández-Pérez *et al*. (2020) and/or extreme environmental conditions can provide more evidences on compromised immunity.

## Oxidative stress and other toxicities

### Oxidative stress

Antibiotics applied in aquaculture production stimulate the generation of excessive endogenous ROS, which subsequently induce oxidative stress. Malondialdehyde (MDA) is the main oxidative product of peroxidised polyunsaturated fatty acids, thus it is an important index of lipid peroxidation. The extent of lipid peroxidation is measured in tissues by quantification of thiobarbituric acid reactive substances (TBARS) expressed as MDA concentration (Nunes *et al*. 2015). The available studies point at decreased MDA and TBARS after exposure to antibiotics at environmental relevant levels (Nunes *et al*. 2015) and toxicological doses (Plhalova *et al*. 2014; Charvatova *et al*. 2015), due to increased antioxidant defence system (Elia *et al*. 2014; Nunes *et al*. 2015) (Table 5), suggesting the ability of some fish species to restore lipid peroxidation following antibiotics treatments.

However, various doses of dietary *in vivo* treatments of antibiotics by using therapeutic (Wang *et al*. 2009; Yonar *et al*. 2011; Yonar 2012; Limbu *et al*. 2018; Zhou *et al*. 2018a; Limbu *et al*. 2019) and both therapeutic and higher doses (Elia *et al*. 2014) increased MDA/TBARS in various fish species. Similarly, *in vivo* exposure of fish to bath antibiotics at environmental relevant levels (Rodrigues *et al*. 2016; Rodrigues *et al*. 2017a; Limbu *et al*. 2018; Rodrigues *et al*. 2018a; Zhou *et al*. 2018b; Rodrigues *et al*. 2018c) and toxicological doses (Han *et al*. 2014; Fonte *et al*. 2016), and NOR bath applied *in vitro* in grass carp (*Ctenopharyngodon idellus*) hepatic cell line (Liu *et al*. 2015), increased MDA/TBARS in various fish species. The increased lipid peroxidation in both *in vivo* and *in vitro* treatments for dietary and bath exposure is related to unbalanced oxidative stress generated by excessive ROS production (Yonar *et al*. 2014; Zhang *et al*. 2015). The induced lipid peroxidation may cause muscle degradation, damage to nervous system and haemolysis, consequently trigger general deterioration of cellular metabolism and eventually cell death (Liu *et al*. 2015), ultimately increase mortality rate as reported before in this work. Such effects are likely to affect the overall aquaculture production sector, considering that exposure to both, dietary and bath doses of antibiotics exert similar effects, although a few studies did not find



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differences in these markers (Bartoskova *et al.,* 2013; Elia *et al.* 2014).

The enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), total antioxidant capacity (T-AOC) and total glutathione (tGSH), together with a non-protein thiol, reduced glutathione (GSH), and the nuclear factor (erythroid-derived 2)-like 2 (NRF2) play significant roles *in vivo* due to their antioxidant functions. They are capable of preventing damage to important cellular components caused by ROS such as free radicals, peroxides and lipid peroxides induced by antibiotics. At the moment, in-depth studies on antibiotics treatments both using dietary and environmental exposure indicate clear effects on antioxidant capacity in several fish species (Table 5). Evidently, feeding fish with diets supplemented with antibiotics by using therapeutic (Yonar *et al.* 2011; Yonar 2012; Andrieu *et al.* 2015; Limbu *et al.* 2018; Pês *et al.* 2018; Zhou *et al.* 2018a), both therapeutic and higher doses (Elia *et al.* 2014) and a series of doses (Andrieu *et al.* 2015) reduced some antioxidant capacity in a dose-dependent manner (Elia *et al.* 2014; Andrieu *et al.* 2015). Similarly, *in vivo* exposure of fish to antibiotics at environmental relevant levels (Rodrigues *et al.* 2016; Limbu *et al.* 2018; Rodrigues *et al.* 2018a; Zhou *et al.* 2018b), toxicological doses (Oliveira *et al.* 2013; Wang *et al.* 2014; Andrieu *et al.* 2015), both environmentally relevant and toxicological concentrations (Plhalova *et al.* 2014; Charvatova *et al.* 2015), and *in vitro* (Liu *et al.* 2015), also reduced some antioxidant capacity in dose-dependent manners (Plhalova *et al.* 2014; Wang *et al.* 2014; Charvatova *et al.* 2015). The inhibited antioxidant capacity in fish, after antibiotics exposure or administration, is due to excess accumulation of free radicals, such as superoxide anion and hydrogen peroxide in different tissues beyond the antioxidant capacity to counteract (Yonar *et al.* 2011; Yonar 2012; Oliveira *et al.* 2013; Wang *et al.* 2014). The reduced antioxidant capacity may hinder detoxification response, subsequently cause lipid peroxidation (Limbu *et al.* 2018), oxidize amino acids and co-factors (Zhang *et al.* 2013), affect enzymes activities (Oliveira *et al.* 2013), induce DNA or RNA damage and enhance toxicity of antibiotics in fish body (Limbu *et al.* 2019). Accordingly, we hypothesize that, the oxidative stress caused by antibiotics reduced the growth performance, damaged the fish body and induced microbiota dysbiosis reported previously herein.

Nevertheless, different dietary doses at therapeutic (Wang *et al.* 2009; Yonar 2012; Rodrigues *et al.* 2017a; Limbu *et al.* 2018; Nakano *et al.* 2018; Limbu *et al.* 2019), excessive dose (Nakano *et al.* 2018), both therapeutic and higher doses (Elia *et al.* 2014) increased some antioxidant capacity in tissue-specific manner (Nakano *et al.* 2018). Likewise, exposure to bath antibiotics at environmental relevant levels (Han *et al.* 2014; Liu *et al.* 2014a; Liu *et al.* 2014b; Rodrigues *et al.* 2016; Yan *et al.* 2016; Qiu *et al.* 2020), toxicological doses (Li *et al.* 2012; Oliveira *et al.* 2013; Wang *et al.* 2014; Liu *et al.* 2014c; Zhang *et al.* 2015), environmental relevant and toxicological concentrations (Bartoskova *et al.,* 2013; Plhalova *et al.* 2014; Charvatova *et al.* 2015), lower than therapeutic doses (Han *et al.* 2014), and *in vitro* (Liu *et al.* 2015), increased some antioxidant capacity in several fish species. In these studies, antibiotics caused dose-dependent (Han *et al.* 2014; Plhalova *et al.* 2014; Wang *et al.* 2014; Charvatova *et al.* 2015; Zhang *et al.* 2015; Qiu *et al.* 2020), tissue-specific (Liu *et al.* 2014a; Liu *et al.* 2014b; Nunes *et al.* 2015; Rodrigues *et al.* 2017a), single and mixture (Li *et al.* 2012; Liu *et al.* 2014c; Qiu *et al.* 2020) and time-related (Liu *et al.* 2014b) effects (Table 5) as mechanisms to reduce ROS production, indicating oxidative stress (Yonar *et al.* 2011; Yonar 2012; Oliveira *et al.* 2013; Nunes *et al.* 2015). Interestingly, both single and combined antibiotics were toxic (Yan *et al.* 2016); sometimes, the latter method rendered stronger toxicity than single exposure (Qiu *et al.* 2020).

Taken together, exposure of fish to dietary and bath antibiotics generates ROS, which cause unbalanced oxidative stress that suppresses antioxidant capacity in various cultured fish species based on species and tissues sampled and antibiotic types and doses. Evidently, *D. rerio* treated with OTC diet increased oxygen consumption rate, indicating stressful condition (Zhou *et al.* 2018a). Occasionally, antibiotics induce collapse and recovery of antioxidant capacity pathways (Oliveira *et al.* 2013; Wang *et al.* 2014), suggesting reversible effects. Accordingly, some of the dietary and bath antibiotics, which decreased (Elia *et al.* 2014; Plhalova *et al.* 2014; Rodrigues *et al.* 2018a; Zhou *et al.* 2018a) or increased (Wang *et al.* 2009; Bartoskova *et al.,* 2013; Elia *et al.* 2014; Plhalova *et al.* 2014; Rodrigues *et al.* 2017a) certain antioxidant capacity, did not do so on other parameters (Rodrigues *et al.* 2018b), suggesting reversible and specific effects depending on fish species and tissues sampled, and antibiotic types and doses. Studies relating the oxidative stress generated to reproductive performances of fish are required in future to support the available limited information (Yan *et al.* 2016).

## Hepatotoxicity and histopathological changes in various fish tissues

The liver of fish and other vertebrates performs digestive, metabolism, storage and detoxification functions. The current available literature insinuate that, the presence of antibiotics in the fish body through medicated feeds and bath treatments impairs liver functions by affecting hepatosomatic index (HSI) in various fish species (Table 6). Various doses of antibiotics induced hepatotoxicity by


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**Table 4** The effects of antibiotics used in global aquaculture on immunity and inflammation response of cultured fish

| Antibiotic | Exposure method and dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline | Dietary; 200 mg $kg^{-1}$ $fish^{-1}$ $day^{-1}$ | Respiratory burst and phagocytosis | *Scophthalmus maximus* | —— | Tafalla *et al*. (1999) |
| Oxytetracycline | Dietary; 60 mg $kg^{-1}$ body weight $day^{-1}$ | Number of plaque-forming cells in | *Cyprinus carpio* | ↓ | Grondel *et al*. (1987) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$ | Antibody production | *Oncorhynchus mykiss* | ↓ | Lundén *et al*. (1998) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$ | Mitogenic response | *Oncorhynchus mykiss* | ↓ | Lundén and Bylund (2000) |
| Oxytetracycline | Exposure; 100 μg $mL^{-1}$ *in vitro* | Chemiluminescence response | *Oncorhynchus mykiss* | ↓ | Lundén *et al*. (2002) |
| Oxytetracycline | Dietary; 4 and 8 mg $g^{-1}$ feed | Hematopoietic-lymphopoietic tissue, | *Sparus aurata* | ↓ | Guardiola *et al*. (2012) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | Leucocyte counts, nitroblue tetrazolium activity, total plasma protein, immunoglobulin levels and phagocytic activity | *Oncorhynchus mykiss* | ↓ | Yonar *et al*. (2011) |
| Oxytetracycline | Dietary; 82.5, 248 and 413 mg $kg^{-1}$ body weight $day^{-1}$ | Hematopoietic-lymphopoietic tissue | *Sander vitreus* | ↓ | Gaikowski *et al*. (2003b) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | Nitroblue tetrazolium activity, total plasma protein, immunoglobulin and phagocytic activity | *Oncorhynchus mykiss* | ↓ | Yonar (2012) |
| Oxytetracycline | Dietary; 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Acid phosphatase and alkaline phosphatase | *Denio rerio* | ↓ | Zhou *et al*. (2018a) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$ | Acid phosphatase and alkaline phosphatase | *Denio rerio* | ↓ | Zhou *et al*. (2018b) |
| Oxytetracycline | Dietary; 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Acid phosphatase and alkaline phosphatase | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$ | Acid phosphatase and alkaline phosphatase | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Dietary; 4 and 8 mg $g^{-1}$ feed | *C3*, *MHC-IIα*, *IL-1β*, *COX-2* and *β-defensin* genes | *Sparus aurata* | ↓ | Guardiola *et al*. (2012) |
| Oxytetracycline | Dietary; 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | β-defensin and hepcidin genes | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$ | β-defensin and hepcidin genes | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Dietary; 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *IL-10* gene | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$ | *IL-10* gene | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 5 μg $L^{-1}$ | *IL-1β* gene | *Danio rerio* | ↓ | López Nadal *et al*. (2018) |
| Oxytetracycline | Exposure; 125 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm and 1500 ppm | *IL-1β* and *mpx* genes | *Danio rerio* | ↑ | Barros-Becker *et al*. (2012) |
| Oxytetracycline | Injection: 50 mg $mL^{-1}$ | Number of granulocytes | *Cyprinus carpio* | ↑ | Rijkers *et al*. (1980) |
| Oxytetracycline | Dietary; 4 and 8 mg $g^{-1}$ feed | Percentage phagocytic cells, phagocytic capacity and respiratory burst | *Sparus aurata* | ↑ | Guardiola *et al*. (2012) |
| Oxytetracycline | Dietary; 200 mg $kg^{-1}$ body weight $day^{-1}$ | Plasma bactericidal activity and total protein concentration | *Scophthalmus maximus* | ↑ | Tafalla *et al*. (1999) |


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**Table 4** (continued)

| Antibiotic | Exposure method and dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight day$^{-}$ | Lysozyme activity | *Oreochromis niloticus* | ↑ | Reda *et al*. (2013) |
| Oxytetracycline | Dietary; 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *IL-1*, *IL-1β*, *TNFα* and *IL-8* genes | *Oreochromis niloticus* | ↑ | Limbu *et al*. (2018) |
| Oxytetracycline | Exposure; 420 ng $L^{-1}$ | *IL-1*, *IL-1β*, *TNFα* and *IL-8* genes | *Oreochromis niloticus* | ↑ | Limbu *et al*. (2018) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$ | Circulating lymphocytes | *Oreochromis niloticus* ♀ × *O. aureus* ♂ | —— | He *et al*. ( 2011a) |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$ | Mitogenic response | *Oncorhynchus mykiss* | ↓ | Lundén and Bylund (2000) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$ | Chemiluminescence response | *Oncorhynchus mykiss* | ↓ | Lundén *et al*. (1999) |
| Florfenicol | Dietary; 10, 30 and 50 mg $kg^{-1}$ body weight $day^{-1}$ | Hematopoietic-lymphopoietic tissue | *Oncorhynchus mykiss* | ↓ | Gaikowski *et al*. (2003a) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$ | Complement component concentrations | *Oreochromis niloticus* ♀ × *O. aureus* ♂ | ↑ | He *et al*. (2011a) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$ | Acid phosphatase and alkaline phosphatase | *Denio rerio* | ↓ | Zhou *et al*. (2018b) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | Acid phosphatase and alkaline phosphatase | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$ | Acid phosphatase and alkaline phosphatase | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | β-defensin and hepcidin genes | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$ | β-defensin and hepcidin genes | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | *IL-10* gene | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | *IL-10* gene | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 0.1, 1, 10 and 100 μg $L^{-1}$ | Lysozyme, *TLR2*, *TLR3*, *TRIF*, *MyD88* and *RAG1* genes | *Danio rerio;* embryos | ↓ | Liu *et al*. (2020b) |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$ | *IL-1*, *IL-1β*, *TNFα* and *IL-8* genes | *Oreochromis niloticus* | ↑ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 260 ng $L^{-1}$ | *IL-1*, *IL-1β*, *TNFα* and *IL-8* genes | *Oreochromis niloticus* | ↑ | Limbu *et al*. (2018) |
| Sulfamethoxazole | Exposure; 0.1, 1, 10 and 100 μg $L^{-1}$ | *IL-1β*, *IFN-c*, *IL-11*, *IL-6* and *TNFα* genes | *Danio rerio;* embryos | | Liu *et al*. (2020b) |
| Sulfadiazine/trimethoprim | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$ | Lysozyme activity, antibody production and circulating leucocyte levels | *Oncorhynchus mykiss* | —— | Lundén and Bylund (2002) |
| Sulfadiazine/trimethoprim | Dietary: 40.5 mg $kg^{-1}$ diet | Agglutinating antibody titres | *Ictalurus punctatus* | ↓ | Wise and Johnson (1998) |
| Sulfadiazine/trimethoprim | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$ | Chemiluminescence response | *Oncorhynchus mykiss* | ↑ | Lundén *et al*. (2002) |
| Sulfadiazine/trimethoprim | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$ | Antibody titres | *Oncorhynchus mykiss* | ↑ | Lundén and Bylund (2002) |
| Oxolinic acid | Dietary; 35 mg $kg^{-1}$ body weight $day^{-1}$ | Antibody production | *Oncorhynchus mykiss* | ↓ | Lundén *et al*. (1998) |


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**Table 4** (continued)

| Antibiotic | Exposure method and dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxolinic acid | Dietary; 35 mg $kg^{-1}$ body weight $day^{-1}$ | Mitogenic response | *Oncorhynchus mykiss* | ↓ | Lundén and Bylund (2000) |
| Oxolinic acid | Dietary; 35 mg $kg^{-1}$ body weight $day^{-1}$ | Chemiluminescence response | *Oncorhynchus mykiss* | ↓ | Lundén *et al.* (2002) |
| Metronidazole | Exposure; 0.1, 0.5, or 2.5 mg $L^{-1}$ | Complement activity, lysozyme activity, bactericidal activity and total white blood cells | *Cyprinus carpio* | ↓ | Han *et al.* (2014) |
| Ciprofloxacin | Exposure; 5 μg $L^{-1}$ | *IL-1β* gene | *Danio rerio* | — | López Nadal *et al.* (2018) |
| β-diketone antibiotics | Exposure; 6.25, 12.5, 25 and 50 mg $L^{-1}$ | Immunoglobulin M | *Danio rerio* | ↑ | Li *et al.* (2016) |
| | Exposure; 6.25, 12.5, 25 and 50 mg $L^{-1}$ | Alkaline phosphatase | *Danio rerio* | ↓↑ | Li *et al.* (2016) |
| | Exposure; 6.25, 12.5, 25 and 50 mg $L^{-1}$ | Lysozyme | *Danio rerio* | ↓↑— | Li *et al.* 2016 |
| | Exposure; 6.25, 12.5, 25 and 50 mg $L^{-1}$ | Complement 3 | *Danio rerio* | ↓ | Li *et al.* 2016 |

The black line indicates no significant effect between treated and control fish, the red line indicates decreased parameters in treated fish compared to control, and blue line indicates increased parameters in treated than control fish. Complement component 3 (*C3*) gene, major histocompatibility complex class II alpha (*MHC-IIα*) gene, interleukin-1 beta (*IL-1β*), cyclooxygenase-2 (*COX-2*), Toll-like receptor 2 (*TLR2*), Toll-like receptor 3 (*TLR3*), (Toll/IL-1 receptor domain containing adaptor inducing IFN-c (*TRIF*), myeloid differentiation factor 88, (*MyD88*), recombination-activating gene 1 (*RAG1*), interferon-c (*IFN-c*), interleukin-11 (*IL-11*), interleukin-6 (*IL-6*), myeloperoxidase (*mpx)* and tumour necrosis factor alpha, (*TNFα*) genes.

decreasing HSI (hepatic atrophy) after dietary therapeutic doses (Refstie *et al.* 2006; Limbu *et al.* 2018; Limbu *et al.* 2020) and bath exposure at environmental relevant level (Limbu *et al.* 2018), indicating liver degenerations (Refstie *et al.* 2006; Reda *et al.* 2013) and severe caloric deficits (Thoolen *et al.* 2010). Moreover, feeding therapeutic dietary doses of OTC (Topic Popovic *et al.* 2012; Trushenski *et al.* 2018) and its excessive amount (Nakano *et al.* 2018) increased HSI (hepatomegaly) in various fish species. Other studies indicated increased liver volume (Madureira *et al.* 2012) and mass (Madureira *et al.* 2011) after exposure to environmental relevant levels in a gender-specific manner and both single and mixture of antibiotics (Madureira *et al.* 2012). These studies suggest swollen liver due to stress (Golden *et al.* 2017) in an attempt to detoxify antibiotics. Consequently, the lack of effects on HSI after therapeutic doses of dietary OTC (Dobšíková *et al.* 2013; Trushenski *et al.* 2018) and acute exposure to tetracycline (Nunes *et al.* 2015) in various fish species was due to effective liver detoxification of the antibiotics. In general, hepatic atrophy or hepatomegaly is undesirable in cultured fish because they cause cirrhosis and hepatotoxicity. The observed physical changes in liver size may affect the ability of the liver to store or produce glucose and plasma proteins.

The amount of circulating proteins and glucose reflects an organism's physiology. Plasma proteins transport lipids, hormones, vitamins and minerals in the body and regulate cellular activity and functioning of the immune system and blood clotting. Imbalances in plasma protein and glucose counts indicate liver damage. Existing studies in the literature show antibiotics reduced plasma proteins levels (Table 6) after dietary doses (Yonar *et al.* 2011; Yonar 2012), even during withdrawal period (Dobšíková *et al.* 2013) and exposure to toxicological doses (Han *et al.* 2014), due to decrease in the mitochondrial protein synthesis caused by deficiencies of cytochrome oxidase C and ATP synthetase enzymes (Reda *et al.* 2013). Further liver damage was evidenced by elevated plasma glucose levels even after withdrawal period post-therapeutic dietary OTC treatment in *C. carpio* (Dobšíková *et al.* 2013) due to glycogenolytic activity of catecholamines and gluconeogenetic effect of glucocorticoids as fish respond to stressful stimuli. Clearly, these studies indicate that, both dietary and bath antibiotics treatments in cultured fish disturb plasma proteins and glucose, indicating hepatotoxicity, which is sustained both during exposure and withdrawal periods.

Hepatotoxicity is further manifested by changes in alanine transaminase (ALT) and aspartate aminotransaminase (AST) enzymes activities in serum, which signal cellular leakage into blood after cytolysis in the liver, highlighting impaired liver cell membrane integrity and function (Han *et al.* 2014). Thus, AST and ALT enzymes are commonly




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**Table 5** Effects of antibiotics on lipid peroxidation and oxidative stress in cultured fish

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline; diet | 75, 150 and 300 mg $kg^{-1}$ body weight $day^{-1}$ | MDA liver | *Cyprinus carpio* | —— | Elia *et al.* (2014) |
| Oxytetracycline; bath | Exposure; 420 ng $L^{-1}$ | MDA muscle and liver | *Denio rerio* | —— | Zhou *et al.* (2018b) |
| Oxytetracycline; bath | Exposure; 420 ng $L^{-1}$ | SOD liver | *Denio rerio* | —— | Zhou *et al.* (2018b) |
| Oxytetracycline, bath | Exposure; 420 ng $L^{-1}$ | MDA liver | *Oreochromis niloticus* | —— | Limbu *et al.* (2018) |
| Oxytetracycline; diet | 75, 150 and 300 mg $kg^{-1}$ body weight $day^{-1}$ | MDA kidney | *Cyprinus carpio* | ↑ | Elia *et al.* (2014) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | MDA | *Oncorhynchus mykiss* | ↑ | Yonar *et al.* (2011) |
| Oxytetracycline, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | MDA | *Oncorhynchus mykiss* | ↑ | Yonar (2012) |
| Oxytetracycline, bath | 0.005, 0.05, 0.5, 5 and 50 mg $L^{-1}$ | TBARS | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2017a) |
| Oxytetracycline, bath | 00.3125, 0.625, 1.25, 2.5 and 5 μg $L^{-1}$ | TBARS | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2017a) |
| Oxytetracycline, bath | 0.0004, 0.004, 0.04, 0.4 and 4 μg $L^{-1}$ | TBARS gills | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2018a) |
| Oxytetracycline, diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | MDA intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline, bath | 420 ng $L^{-1}$ | MDA intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | MDA intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2019) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | MDA liver | *Denio rerio* | —— | Zhou *et al.* (2018b) |
| Sulfamethoxazole, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | MDA intestine | *Oreochromis niloticus* | —— | Limbu *et al.* (2018) |
| Sulfamethoxazole, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | MDA liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole, bath | 260 ng $L^{-1}$ | MDA intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | MDA intestine | *Denio rerio* | ↑ | Zhou *et al.* (2018a) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | MDA intestine | *Denio rerio* | ↑ | Zhou *et al.* (2018b) |
| Norfloxacin; bath | 0.1, 1, 10 and 30 mg $L^{-1}$ | TBARS | *Denio rerio* | —— | Bartoskova et al. (2013) |
| Norfloxacin; bath | 0.0001, 0.1, 1.0, 5.0 and 10.0 mg $L^{-1}$ | TBARS | *Cyprinus carpio* | ↓ | Charvatova *et al.* (2015) |
| Enrofloxacin; diet | 1 g $kg^{-1}$ of food | LPO | *Pangasianodon hypophthalmus* | ↑ | Wang *et al.* (2009) |
| Enrofloxacin; bath | 50, 100 and 200 mg $mL^{-1}$ | MDA | *Ctenopharyngodon idellus* cells | ↑ | Liu *et al.* (2015) |
| Tetracycline; bath | 5, 50 and 500 ng $L^{-1}$ | TBARS | *Gambusia holbrooki* | ↓ | Nunes *et al.* (2015) |
| Ciprofloxacin; bath | 0.7, 100, 650, 1100 and 3000 μg $L^{-1}$ | TBARS | *Danio rerio* | ↓ | Plhalova *et al.* (2014) |
| Metronidazole; bath | 0.1, 0.5, or 2.5 mg $L^{-1}$ | MDA | *Cyprinus carpio* | ↑ | Han *et al.* (2014) |
| Erythromycin; bath | 0.001, 0.01, 0.1, 1,10 mg $L^{-1}$ and 0.05, 0.1, 0.2, 0.4 and 0.8 μg $L^{-1}$ | MDA | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2016) |
| Cefalexin; bath | 1.3, 2.5, 5 and 10 mg $L^{-1}$ | LPO | *Pomatoschistus microps* | ↑ | Fonte *et al.* (2016) |
| Oxytetracycline; diet | 75, 150 and 300 mg $kg^{-1}$ body weight $day^{-1}$ | GPx | *Cyprinus carpio* | —— | Elia *et al.* (2014) |
| Oxytetracycline, bath | 00.3125, 0.625, 1.25, 2.5 and 5 μg $L^{-1}$ | GR | *Oncorhynchus mykiss* | —— | Rodrigues *et al.* (2017a) |
| Oxytetracycline, bath | 420 ng $L^{-1}$ | SOD liver | *Denio rerio* | —— | Zhou *et al.* (2018b) |
| Oxytetracycline; bath | 0.005, 0.05, 0.5, 5 and 50 mg $L^{-1}$ | GST gills acute | *Oncorhynchus mykiss* | —— | Rodrigues *et al.* (2018b) |


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**Table 5** (continued)

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline; bath | 0.005, 0.05, 0.5, 5 and 50 mg $L^{-1}$ and 0.3125, 0.625, 1.25, 2.5 and 5.0 μg $L^{-1}$ | GST liver acute and chronic | *Oncorhynchus mykiss* | —— | Rodrigues *et al.* (2018b) |
| Oxytetracycline; bath | 0.0004, 0.004, 0.04, 0.4 and 4 μg $L^{-1}$ | CAT, GPx and GRed gills and liver | *Sparus aurata* | —— | Rodrigues *et al.* (2018a) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | SOD, CAT, GPx and GSH in blood, liver, kidney, spleen and heart tissues | *Oncorhynchus mykiss* | ↓ | Yonar *et al.* (2011) |
| Oxytetracycline, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | SOD, CAT, GPx and GSH in the blood, liver, kidney and spleen | *Oncorhynchus mykiss* | ↓ | Yonar (2012) |
| Oxytetracycline; diet | 75, 150 and 300 mg $kg^{-1}$ body weight $day^{-1}$ | SOD, dose-dependent | *Cyprinus carpio* | ↓ | Elia *et al.* (2014) |
| Oxytetracycline; bath | 1, 10, 25, 50 and 100 mg $L^{-1}$ | CAT | *Danio rerio* | ↓ | Oliveira *et al.* (2013) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | SOD and GSH intestine | *Denio rerio* | ↓ | Zhou *et al.* (2018b) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | SOD muscle | *Denio rerio* | ↓ | Zhou *et al.* (2018b) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | T-AOC intestine and liver | *Oreochromis niloticus* | ↓ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | T-AOC intestine and liver | *Oreochromis niloticus* | ↓ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | 0.0004, 0.004, 0.04, 0.4 and 4 μg $L^{-1}$ | GST gills chronic | *Sparus aurata* | ↓ | Rodrigues *et al.* (2018a) |
| Oxytetracycline; bath | 0.1, 10, 10000 mg $L^{-1}$ | GST and CAT chronic | *Denio rerio* | ↓ | Almeida *et al.* (2019) |
| Oxytetracycline; diet | 0.1 g kg $diet^{-1}$ | SOD, GPx and GST | *Rhamdia quelen* | ↓ | Pês *et al.* (2018) |
| Oxytetracycline; diet | 75, 150 and 300 mg $kg^{-1}$ body weight $day^{-1}$ | CAT, GPx, GR and GSH liver | *Cyprinus carpio* | ↑ | Elia *et al.* (2014) |
| Oxytetracycline, bath | 00.3125, 0.625, 1.25, 2.5 and 5 μg $L^{-1}$ | CAT and GPx gills | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2017a) |
| Oxytetracycline, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | GST | *Oncorhynchus mykiss* | ↑ | Yonar (2012) |
| Oxytetracycline; bath | 1, 10, 25, 50 and 100 mg $L^{-1}$ | GST | *Danio rerio* | ↑ | Oliveira *et al.* (2013) |
| Oxytetracycline, diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | tGSH | *Oncorhynchus kisutch* | ↑ | Nakano *et al.* (2018) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | SOD and GST intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; diet | Exposure; 420 ng $L^{-1}$ | *NRF2* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | Exposure; 420 ng $L^{-1}$ | *NRF2* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *SOD1* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | *SOD1* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | SOD and GST intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; bath | 0.005, 0.05, 0.5, 5 and 50 mg $L^{-1}$ and 0.3125, 0.625, 1.25, 2.5 and 5.0 μg $L^{-1}$ | GST gills chronic | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2018b) |
| Oxytetracycline; bath | 0.005, 0.05, 0.5, 5 and 50 mg $L^{-1}$ and 0.3125, 0.625, 1.25, 2.5 and 5.0 μg $L^{-1}$ | GST gills chronic | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2018b) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | *NRF2* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | SOD and GST intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2019) |
| Tetracycline; bath | 0.01, 1 and 100 μg $L^{-1}$ | ROS | *Danio rerio* | ↑ | Qiu *et al.* (2020) |


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**Table 5** (continued)

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | SOD and GSH | *Denio rerio* | —— | Zhou *et al.* (2018a) |
| Sulfamethoxazole; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *NRF2* gene intestine and liver | *Oreochromis niloticus* | ↓ | Limbu *et al.* (2018) |
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | T-AOC intestine and liver | *Oreochromis niloticus* | ↓ | Limbu *et al.* (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | SOD and GSH | *Denio rerio* | ↓ | Zhou *et al.* (2018b) |
| Sulfamethoxazole; bath | 0.016, 0.08, 0.4, 2, 10 and 50 mg $L^{-1}$ | GST and SOD | *Carassius auratus* | ↑ | Li *et al.* (2012) |
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | SOD and GST intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | *SOD1* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | *SOD1* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | SOD and GST intestine | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamethoxazole; bath | 2, 20 and 200 μg $L^{-1}$ | CAT and SOD | *Danio rerio* | ↑ | Yan *et al.* (2016) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | *NRF2* gene intestine and liver | *Oreochromis niloticus* | ↑ | Limbu *et al.* (2018) |
| Sulfamonomethoxine; bath | 0.01, 1 and 100 μg $L^{-1}$ | ROS | *Danio rerio* | ↑ | Qiu *et al.* (2020) |
| Norfloxacin; bath | 0.1, 1, 10 and 30 mg $L^{-1}$ | GR | *Denio rerio* | —— | Bartoskova et al. (2013) |
| Norfloxacin; bath | 0.0001, 0.1, 1.0, 5.0 and 10.0 mg $L^{-1}$ | GPx | *Cyprinus carpio* | ↓ | Charvatova *et al.* (2015) |
| Norfloxacin; bath | 0.1, 1, 10 and 30 mg $L^{-1}$ | GPx, GST and CAT | *Denio rerio* | ↑ | Bartoskova et al. (2013) |
| Norfloxacin; bath | 0.0032, 0.016, 0.08, 0.4, 2 and 10 mg $L^{-1}$ | GST | *Carassius auratus* | ↑ | Liu *et al.* (2014c) |
| Norfloxacin + Sulfamethoxazole; bath | 0.0016 + 0.008, 0.008 + 0.04, 0.04 + 0.2, 0.2 + 1, 1 + 5 and 5 + 25 mg $L^{-1}$ | GST and SOD | *Carassius auratus* | ↑ | Liu *et al.* (2014c) |
| Norfloxacin; bath | 2, 20 and 200 μg $L^{-1}$ | CAT and SOD | *Danio rerio* | ↑ | Yan *et al.* (2016) |
| Norfloxacin; bath | 0.0001, 0.1, 1.0, 5.0 and 10.0 mg $L^{-1}$ | GST and GR | *Cyprinus carpio* | ↑ | Charvatova *et al.* (2015) |
| Norfloxacin; bath | 12.5, 25, 50, 100 and 200 μg $mL^{-1}$ | SOD | *Ctenopharyngodon idella* cells | ↑ | Liu *et al.* (2015) |
| Norfloxacin; bath | 0.24, 1.2 and 6 mg $L^{-1}$ | *GST* gene | *Xiphophorus helleri* | ↑ | Liang *et al.* (2015) |
| Erythromycin; bath | 0.001, 0.010, 0.100, 1, 10 mg $L^{-1}$ | GPx gills and liver acute | *Oncorhynchus mykiss* | —— | Rodrigues *et al.* (2016) |
| Erythromycin; bath | 0.001, 0.01, 0.1, 1 and 10 mg $L^{-1}$ | GST gills and liver acute | *Oncorhynchus mykiss* | —— | Rodrigues *et al.* (2018c) |
| Erythromycin; bath | 0.001, 0.01, 0.1, 1 and 10 mg $L^{-1}$ | CAT dose in gills during acute exposure | *Oncorhynchus mykiss* | ↓ | Rodrigues *et al.* (2016) |
| Erythromycin; bath | 0.05, 0.1, 0.2, 0.4 and 0.8 μg $L^{-1}$ | CAT gills during chronic exposure | *Oncorhynchus mykiss* | ↓ | Rodrigues *et al.* (2016) |
| Erythromycin; bath | 0.001, 0.01, 0.1, 1,10 mg $L^{-1}$ and 0.05, 0.1, 0.2, 0.4 and 0.8 μg $L^{-1}$ | GPx and GR in gills | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2016) |
| Erythromycin; bath | 4, 20 and 100 μg $L^{-1}$ | SOD | *Carassius auratus* | ↑ | Liu *et al.* (2014a) |
| Erythromycin; bath | 0.05, 0.10, 0.20, 0.40 and 0.80 μg $L^{-1}$ | GST gills chronic | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al.* (2018c) |
| Enrofloxacin; diet | 1 g $kg^{-1}$ of food | GST and tGSH | *Pangasianodon hypophthalmus* | —— | Wang *et al.* (2009) |


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**Table 5** (continued)

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Enrofloxacin; diet | 30, 75, 150, 300 mg $kg^{-1}$ body weight $day^{-1}$ | CAT, dose- and time-dependent | *Oreochroms niloticus* | ↓ | Andrieu *et al.* (2015) |
| Enrofloxacin; bath | 12.5, 25, 50, 100 and 200 μg $mL^{-1}$ | T-AOC | *Ctenopharyngodon idellus* cells | ↓ | Liu *et al.* (2015) |
| Enrofloxacin; diet | 1 g $kg^{-1}$ of food | CAT | *Pangasianodon hypophthalmus* | ↑ | Wang *et al.* (2009) |
| Enrofloxacin; bath | 12.5, 25, 50, 100 and 200 μg $mL^{-1}$ | ROS | *Ctenopharyn godon idellus* cells | ↑ | Liu *et al.* (2015) |
| Enrofloxacin; bath | 0.01, 1 and 100 μg $L^{-1}$ | ROS | *Danio rerio* | ↑ | Qiu *et al.* (2020) |
| Ciprofloxacin; bath | 0.7, 100, 650, 1100 and 3000 μg $L^{-1}$ | CAT | *Danio rerio* | — | Plhalova *et al.* (2014) |
| Ciprofloxacin; diet | 15, 33.6, 75, 150 mg $kg^{-1}$ body weight $day^{-1}$ | CAT and GR | *Oreochroms niloticus* | — | Andrieu *et al.* (2015) |
| Ciprofloxacin; bath | 0.7, 100, 650, 1100 and 3000 μg $L^{-1}$ | GPx and GR | *Danio rerio* | ↓ | Plhalova *et al.* (2014) |
| Ciprofloxacin; bath | 0.7, 100, 650, 1100 and 3000 μg $L^{-1}$ | GST | *Danio rerio* | ↑ | Plhalova *et al.* (2014) |
| Tetracycline; bath | 5, 50 and 500 ng $L^{-1}$ | CAT and GST | *Gambusia holbrooki* | ↑ | Nunes *et al.* (2015) |
| Tetracycline; bath | 2, 10, 20, 200, 2000 and 20000 μg $L^{-1}$ | ROS | *Denio rerio* | ↑ | Zhang *et al.* (2015) |
| Tetracycline; bath | 2, 10, 20, 200, 2000 and 20000 μg $L^{-1}$ | *SOD* and *GST* genes | *Denio rerio* | ↑ | Zhang *et al.* (2015) |
| Roxithromycin; bath | 4, 20 and 100 μg $L^{-1}$ | SOD | *Carassius auratus* | ↑ | Liu *et al.* (2014b) |
| †β-diketone antibiotics; bath | 2.34, 9.38 and 37.5 mg $L^{-1}$ | SOD | *Danio rerio* | ↓ | Wang *et al.* (2014) |
| †β-diketone antibiotics; bath | 2.34, 9.38 and 37.5 mg $L^{-1}$ | GSH lower concentration | *Danio rerio* | ↑ | Wang *et al.* (2014) |
| Cefotaxime sodium; bath | 0.01, 1 and 100 μg $L^{-1}$ | ROS | *Danio rerio* | ↑ | Qiu *et al.* (2020) |
| Amoxicillin; bath | 1, 10, 25, 50 and 100 mg $L^{-1}$ | CAT | *Danio rerio* | ↓ | Oliveira *et al.* (2013) |
| Amoxicillin; bath | 1, 10, 25, 50 and 100 mg $L^{-1}$ | GST | *Danio rerio* | ↑ | Oliveira *et al.* (2013) |
| Metronidazole; bath | 0.1, 0.5, or 2.5 mg $L^{-1}$ | CAT, SOD and GSH | *Cyprinus carpio* | ↑ | Han *et al.* (2014) |

The black line indicates no significant effect between treated and control fish, the red line indicates decreased parameters in treated fish compared to control, and blue line indicates increased parameters in treated than control fish. Malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), lipid peroxidation levels (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), total antioxidant capacity (T-AOC) and total glutathione (tGSH), reduced glutathione (GSH), nuclear factor (erythroid-derived 2)-like 2 (NRF2) and reactive oxygen species (ROS)

used to detect hepatotoxicity due to xenobiotics exposure (Saravanan *et al.* 2012). Presently, studies reporting hepatotoxicity of antibiotics based on AST and ALT are limited (Table 6). Available results indicate a biphasic hepatotoxicity trend in gill, liver and muscle (Ambili *et al.* 2013) after feeding therapeutic dose of dietary OTC, mainly due to insufficient detoxification mechanisms to prevent the toxicity action of the antibiotic or failure of damaged liver cells to synthesize AST and ALT proteins (Saravanan *et al.* 2012). Conversely, increased hepatotoxicity occurred in several fish species after dietary doses of therapeutic (Reda *et al.* 2013; Limbu *et al.* 2020) and excessive (Nakano *et al.* 2018) and bath exposure to toxicological doses (Han *et al.* 2014), due to the ability of antibiotics to accumulate or bind to different cells. The cells are eventually damaged and disintegrated releasing AST and ALT into blood circulation, indicating hepatotoxicity. In general, antibiotics induce hepatotoxicity due to the generation of oxidative stress depending on their types and hepatotoxicity marker. Evidently, therapeutic OTC dose did not affect ALT, while therapeutic FFC concentration increased AST in *O. niloticus* (Reda *et al.* 2013). The induced hepatotoxicity in cultured fish may affect digestive, metabolism and detoxification functions of the liver.


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**Table 6** The effects of antibiotics on hepatotoxicity and histological damages in cultured fish

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline; diet | 75 mg $kg^{-1}$ body weight $day^{-1}$ | HSI | *Cyprinus carpio* | —— | Dobšíková *et al*. (2013) |
| Oxytetracycline; diet | 16 or 80 mg $kg^{-1}$ body weight $day^{-1}$ | HSI | *Ictalurus punctatus, Oreochromis niloticus, Oncorhynchus mykiss* | —— | Trushenski *et al*. (2018) |
| Oxytetracycline; diet | 3 g $kg^{-1}$ diet | HSI | *Salmo salar* | ↓ | Refstie *et al*. (2006) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | HSI | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline; bath | 260 ng $L^{-1}$ | | | | |
| Oxytetracycline; diet | 82.8 mg $kg^{-1}$ body weight $day^{-1}$ | HSI | *Morone saxatilis* ♂ × *M. chrysops*♀ | ↑ | Topic Popovic *et al*. (2012) |
| Oxytetracycline; diet | 16 or 80 mg $kg^{-1}$ body weight $day^{-1}$ | HSI lower dose | *Morone chrysops* × *M. saxatilis* | ↑ | Trushenski *et al*. (2018) |
| Oxytetracycline; diet | 75 mg $kg^{-1}$ body weight $day^{-1}$ | HSI | *Oncorhynchus kisutch* | ↑ | Nakano *et al*. (2018) |
| Tetracycline; bath | 5, 50 and 500 ng $L^{-1}$ | HSI | *Gambusia holbrooki* | —— | Nunes *et al*. (2015) |
| Sulfamethoxazole; bath | 533 μg $L^{-1}$ | Liver volume | *Denio rerio* | ↑ | Madureira *et al*. (2012) |
| Trimethoprim; bath | 157 μg $L^{-1}$ | | | | |
| Sulfamethoxazole; bath | 533 μg $L^{-1}$ | Hepatic mass | *Denio rerio* | ↑ | Madureira *et al*. (2011) |
| Trimethoprim; bath | 157 μg $L^{-1}$ | | | | |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | Plasma protein | *Oncorhynchus mykiss* | ↓ | Yonar *et al*. (2011) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | Plasma protein | *Oncorhynchus mykiss* | ↓ | Yonar (2012) |
| Oxytetracycline; diet | 75 mg $kg^{-1}$ body weight $day^{-1}$ | Plasma protein | *Cyprinus carpio* | ↓ | Dobšíková *et al*. (2013) |
| Metronidazole; bath | 0.1, 0.5, or 2.5 mg $L^{-1}$ | Plasma protein | *Cyprinus carpio* | ↓ | Han *et al*. (2014) |
| Oxytetracycline; diet | 75 mg $kg^{-1}$ body weight $day^{-1}$ | Plasma glucose | *Cyprinus carpio* | ↑ | Dobšíková *et al*. (2013) |
| Oxytetracycline; bath | 80 mg $L^{-1}$ | AST and ALT time-dependent in gill, liver and muscle | *Labeo rohita* | ↓↑ | Ambili *et al*. (2013) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ diet | ALT | *Oreochromis niloticus* | ↑ | Reda *et al*. (2013) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | ALT | *Oncorhynchus kisutch* | ↑ | Nakano *et al*. (2018) |
| Florfenicol; diet | 100 mg $kg^{-1}$ diet | AST | *Oreochromis niloticus* | —— | Reda *et al*. (2013) |
| Metronidazole; bath | 0.1, 0.5, or 2.5 mg $L^{-1}$ | AST and ALT liver | *Cyprinus carpio* | ↑ | Han *et al*. (2014) |
| Oxytetracycline; diet | 82.8 mg $kg^{-1}$ body weight | Liver | *Morone saxatilis* ♂ × *M. chrysops*♀ | —— | Topic Popovic *et al*. (2012) |
| Oxytetracycline; diet | 3 g $kg^{-1}$ diet | Intestine histopathological changes | *Salmo salar* | —— | Bakke-McKellep *et al*. (2007) |
| Oxytetracycline; diet | 20 mg $kg^{-1}$ body weight $day^{-1}$ | Serum albumin | *Oncorhynchus mykiss* | ↓ | Maklakova *et al*. (2011) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ diet | Creatinine | *Oreochromis niloticus* | ↓ | Reda *et al*. (2013) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Goblet cells | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | Goblet cells | *Denio rerio* | ↓ | Zhou *et al*. (2018b) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Muscularis thickness | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Villi width | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Villi height | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Villi width | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | | | | |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Muscularis thickness | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | | | | |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *CLDN 3* mRNA expression | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *ZO 1* mRNA expression | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2019) |


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**Table 6** (continued)

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *CLDN 1*, *3* and *4*, Occludin and *ZO 1* mRNA expression | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Oxytetracycline; bath | 420 ng $L^{-1}$ | | | | |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ diet | Liver and kidney damages | *Oreochromis niloticus* | ↑ | Reda *et al*. (2013) |
| Oxytetracycline; diet | 20 mg $kg^{-1}$ body weight $day^{-1}$ | Granulopenia, lipoid assemblage | *Oncorhynchus mykiss* | ↑ | Maklakova *et al*. (2011) |
| Oxytetracycline; bath | 0.005, 0.05, 0.5, 5, 50 mg $L^{-1}$ and 0.3125, 0.625, 1.25, 2.5, 5 μg $L^{-1}$ | Gills and liver damages | *Oncorhynchus mykiss* | ↑ | Rodrigues *et al*. (2017b) |
| Florfenicol; diet | 10, 20, 40, or 100 mg $kg^{-1}$ body weight $day^{-1}$ | Liver and other organs | *Ictalurus punctatus* | — | Gaunt *et al*. (2003) |
| Florfenicol; diet | 15, 45 and 75 mg $kg^{-1}$ body weight $day^{-1}$ | Skin, muscle and intestine tissues | *Perca flavescens* | — | Bowker *et al*. (2013) |
| Florfenicol; diet | 5 mg $kg^{-1}$ body weight $day^{-1}$ | Creatinine | *Oreochromis niloticus* | ↓ | Reda *et al*. (2013) |
| Florfenicol; diet | 5 mg $kg^{-1}$ body weight $day^{-1}$ | Liver and kidney damages | *Oreochromis niloticus* | ↑ | Reda *et al*. (2013) |
| Florfenicol; diet | 15, 45 and 75 mg $kg^{-1}$ body weight $day^{-1}$ | Gills, liver and kidney damages | *Oreochromis* spp | ↑ | Gaikowski *et al*. (2013) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | Goblet cells | *Denio rerio* | ↓ | Zhou *et al*. (2018b) |
| Sulfamethoxazole; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | Villi width | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | | | | |
| Sulfamethoxazole; bath | 533 μg $L^{-1}$ | Nuclear υ of hepatocytes | *Denio rerio* | ↑ | Madureira *et al*. (2012) |
| Trimethoprim; bath | 157 μg $L^{-1}$ | | | | |
| Sulfamethoxazole; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | Muscularis thickness | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | | | | |
| Sulfamethoxazole; diet | 82.5 mg $kg^{-1}$ body weight $day^{-1}$ | *CLDN 1*, *3* and *4*, Occludin and *ZO 1* mRNA expression | *Oreochromis niloticus* | ↓ | Limbu *et al*. (2018) |
| Sulfamethoxazole; bath | 260 ng $L^{-1}$ | | | | |
| †β-diketone antibiotics; bath | 4.69, 9.38, 18.75 and 37.5 mg $L^{-1}$ | Heart tissue histopathological changes | *Danio rerio* | ↑ | Yin *et al*. (2014) |
| †β-diketone antibiotics; bath | 4.69, 9.38, 18.75 and 37.5 mg l-1 37.5 mg $L^{-1}$ | Creatine kinase and creatine concentration | *Danio rerio* | ↑ | Yin *et al*. (2014) |
| Tetracycline; bath | 5, 50 and 500 ng $L^{-1}$ | Histological alterations in gills | *Gambusia holbrooki* | ↑ | Nunes *et al*. (2015) |
| Sulfisoxazole, sulfamerazine, sulfamerazine/ sulfaguanidine; diets | 200 mg $kg^{-1}$ body weight $day^{-1}$ | Kidneys and other organs | *Salvelinus fontinalis, Salmo truta, Salmo gairdneri* | — | Snieszko and Wood (1955) |
| Gentamycin; injection | 10 mg $mL^{-1}$ | Renal failure | *Danio rerio* | ↑ | Hentschel *et al*. (2005) |

The black line indicates no significant effect between treated and control fish, the red line indicates decreased parameters in treated fish compared to control, and blue line indicates increased parameters in treated than control fish. Hepatosomatic index (HSI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Claudin 3 (*CLDN 3*) and Zona occludens 1 (*ZO 1*).

Histopathological effects provide a quick diagnosis to detect abnormalities in various fish tissues and organs after antibiotics exposure. Accordingly, various dietary doses of antibiotics caused multiple histopathological damages, particularly in vital organs such as liver (Reda *et al*. 2013; Rodrigues *et al*. 2019), kidney (Gaikowski *et al*. 2013; Reda *et al*. 2013), gills (Rodrigues *et al*. 2019) and intestine (Limbu *et al*. 2018; Limbu *et al*. 2019), due to oxidative stress and their toxicity (Table 6). Similar histopathological effects were reported for different doses of bath treatments in liver (Maklakova *et al*. 2011; Madureira *et al*. 2012; Rodrigues *et al*. 2017b), intestine (Limbu *et al*. 2018; Limbu *et al*. 2019), heart (Yin *et al*. 2014), gills (Nunes *et al*. 2015) and injections, which damaged kidney (Hentschel *et al*. 2005) and liver, intestine, spleen and gallbladder (Maklakova *et al*. 2011), in various fish species (Table 6). Some of the antibiotics effects on fish species were gender-specific (Madureira *et al*. 2012). The histopathological damages in




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various tissues suggest that, antibiotics inhibit somatic cell synthesis, cause proliferation arrest of normal and malignant cells of epithelial origin (Bakke-McKellep *et al.* 2007) and impair nutrients absorption, digestion and metabolism (Limbu *et al.* 2018; Limbu *et al.* 2019) and excretion (Reda *et al.* 2013). The liver damage observed also worsens the hepatotoxicity and body malformation induced by antibiotics as reported before in this review.

In summary, feeding, exposing or injecting fish with antibiotics cause various histopathological effects in vital organs such as liver, kidney, gills and intestine due to oxidative stress and their toxicity, suggesting impaired nutrition metabolism, gaseous exchange, osmoregulation and digestive functions. These results imply severe effects on fish physiology because both exposure to acute doses and chronic treatment of antibiotics caused deleterious histological effects (Rodrigues *et al.* 2019), although a few antibiotics did not induce significant organ changes (Snieszko & Wood 1955; Gaunt *et al.* 2003; Bakke-McKellep *et al.* 2007; Topic Popovic *et al.* 2012; Bowker *et al.* 2013), highlighting antibiotic and fish species-specific damage. Since most cultured fish are more susceptible to secondary infections, the histopathological alterations may affect fish haematological parameters.

## Haematological parameters

Haematological parameters provide important information on the health of cultured fish after antibiotics exposure. The available scientific literature demonstrates impaired haematological parameters (Table 7), by decreasing their levels after treatment with various doses of dietary (Lundén *et al.* 1998; Yonar 2012; Ambili *et al.* 2013) and bath (Han *et al.* 2014; Nwani *et al.* 2014) antibiotics, due to their toxic effects on various organs and tissues (Yonar 2012; Nwani *et al.* 2014). Antibiotics cause blood cells swelling (Ambili *et al.* 2013), release of immature and damage erythrocytes (Ambili *et al.* 2013; Nwani *et al.* 2014), decrease in erythrocytes life span (Ambili *et al.* 2013), inhibit mitochondrial and cytoplasmic protein synthesis (Lundén *et al.* 1998), decrease in neutrophils secretion from granulocytes (Han *et al.* 2014) and inhibit haemoglobin biosynthesis (Nwani *et al.* 2014). The inhibition of haematological parameters sustains the toxic effects of antibiotics, which damage various tissues, suppress immunity and limit oxygen carrying capacity of fish blood (Nwani *et al.* 2014). The limited oxygen carrying capacity may cause fatal outcomes such as impaired aerobic metabolism in cultured fish. Plausible, inhibited haematological parameters were responsible for the lipid peroxidation, histopathological damage and suppressed immunity in the previous sections. The adverse effects, ultimately impair fish health with possible fatal outcomes, considering that toxic effects of antibiotics on haematological parameters prevail even after withdrawal periods (Han *et al.* 2014).

Notwithstanding the above results, a few antibiotics elevated haematological parameters (Ambili *et al.* 2013; Nwani *et al.* 2014), suggesting increased disease-fighting cells as a protective response of fish body. Moreover, the haematological parameters in both studies (Ambili *et al.* 2013; Nwani *et al.* 2014) indicated mixed trends, pointing to failure and recovery of haematological parameters due to insufficient synthesis of haemoglobin and microcytic anaemia following antibiotics application (Kumar *et al.* 2013) and compensation mechanism for impaired oxygen uptake caused by tissue damage and high percentage of circulating immature erythrocytes (Ambili *et al.* 2013). Altogether, exposure of cultured fish to dietary and bath antibiotics distresses haematological parameters, suggesting sustained toxic effects, which affect health and aerobic metabolism of fish. However, studies relating the haematological effects to physiological and pathological status are currently missing.

## Nutrients metabolism

Nutrients metabolism provide energy for expenditure in various activities including stress in the fish body (Limbu *et al.* 2020). Therefore, changes in nutrients metabolism in fish after antibiotics signal adverse effects. Lactate dehydrogenase (LDH) is potential biomarkers for assessing toxicity during xenobiotics exposure. Numerous scientific researches indicate changes in nutrients metabolism in cultured fish following dietary and bath exposure, both *in vivo* and *in vitro*. As a matter of fact, the exposure of different fish species to therapeutic doses of dietary (Dobšíková *et al.* 2013; Limbu *et al.* 2018) and environmental relevant level (Limbu *et al.* 2018) and toxicological concentrations (Ambili *et al.* 2013; Oliveira *et al.* 2013; Rodrigues *et al.* 2018b) of antibiotics *in vivo* studies, together with *in vitro* exposure of *C. idellus* hepatic cell line to NOR (Liu *et al.* 2015), increased LDH activity due high energy demand under toxic stress. Such an increase in LDH emanates from conversion of pyruvate into lactate under anaerobic conditions (Ambili *et al.* 2013; Limbu *et al.* 2018). Evidently, both dietary and bath OTC and SMZ in *O. niloticus* inhibited aerobic glycolysis, enhanced gluconeogenesis and suppressed lipogenesis and fatty acid β-oxidation in liver and intestine due to excessive ROS generation, which increased energy expenditure (Limbu *et al.* 2018). Moreover, OTC bath also reduced lipid levels and increased energy consumption in *D. rerio* (Almeida *et al.* 2019), and single and combined antibiotics disrupted several nutrients metabolic pathways (Qiu *et al.* 2020), highlighting physiological stress in treated fish. Antibiotics may also impair nutrients metabolism through reduced digestive enzymes activities and nutrients digestibility, microbiota dysbiosis, liver


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**Table 7** Effects of antibiotics on haematological parameters in cultured fish

| Antibiotic/exposure method | Dose | Parameter | Fish species | Effect | Reference |
|---|---|---|---|---|---|
| Oxytetracycline; bath | 5 μg $L^{-1}$ | Neutrophil and macrophage | *Danio rerio* | — | López Nadal *et al.* (2018) |
| Oxytetracycline; diet | 35 mg $kg^{-1}$ body weight $day^{-1}$ | WBC, especially lymphocytes | *Oncorhynchus mykiss* | ↓ | Lundén *et al.* (1998) |
| Oxytetracycline; diet | 100 mg $kg^{-1}$ body weight $day^{-1}$ | Haematocrit | *Oncorhynchus mykiss* | ↓ | Yonar (2012) |
| Oxytetracycline; diet | 80 mg $L^{-1}$ | RBC count | *Labeo rohita* | ↓ | Ambili *et al.* (2013) |
| Oxytetracycline; diet | 80 mg $L^{-1}$ | WBC | *Labeo rohita* | ↑ | Ambili *et al.* (2013) |
| Oxytetracycline; diet | 80 mg $L^{-1}$ | Hb, haematocrit, MCV, MCH and MCHC | *Labeo rohita* | ↓↑ | Ambili *et al.* (2013) |
| Chloramphenicol; bath | 2.5, 5 and 10 mg $L^{-1}$ | Monocytes, eosinophils and basophils | *Clarias gariepinus* | — | Nwani *et al.* (2014) |
| Chloramphenicol; bath | 2.5, 5 and 10 mg $L^{-1}$ | Haemoglobin (Hb) and MCV | *Clarias gariepinus* | ↓ | Nwani *et al.* (2014) |
| Chloramphenicol; bath | 2.5, 5 and 10 mg $L^{-1}$ | Lymphocytes | *Clarias gariepinus* | ↑ | Nwani *et al.* (2014) |
| Chloramphenicol; bath | 2.5, 5 and 10 mg $L^{-1}$ | RBC, WBC, MCH, MCHC and neutrophils | *Clarias gariepinus* | ↓↑ | Nwani *et al.* (2014) |
| Metronidazole; bath | 0.1, 0.5, or 2.5 mg $L^{-1}$ | Total WBC count, complement activity, lysozyme activity and bactericidal activity | *Cyprinus carpio* | ↓ | Han *et al.* (2014) |
| Ciprofloxacin; bath | 5 μg $L^{-1}$ | Neutrophil and macrophage | *Danio rerio* | — | López Nadal *et al.* (2018) |
| Oxolinic acid; diet | 35 mg $kg^{-1}$ body weight $day^{-1}$ | WBC, especially lymphocytes | *Oncorhynchus mykiss* | ↓ | Lundén *et al.* (1998) |

The black line indicates no significant effect between treated and control fish, the red line indicates decreased parameters in treated fish compared to control, and blue line indicates increased parameters in treated than control fish. White blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), mean cellular volume (MCV), mean cellular haemoglobin (MCH) and mean cellular Hb concentration (MCHC).

histopathology and hepatotoxicity as indicated previously in this review.

Taken together, antibiotics impair nutrients metabolism by increasing energy demand through their toxicity and excessive ROS generation in fish body. A few limited doses of bath antibiotics (Rodrigues *et al.* 2018a; Rodrigues *et al.* 2018c; Almeida *et al.* 2019) in *in vivo* studies and *in vitro* after NOR bath exposure in tilapia head kidney-derived macrophages (THKDM) cells (Soto *et al.* 2010), which did not show effect on LDH, might be caused by biphasic shifts in the measured parameter (Dobšíková *et al.* 2013). This calls for further studies using proteomics to verify the transcription results obtained.

## Neurotoxicity effects

Acetylcholinesterase (AChE) is an enzyme participating in cholinergic neurotransmission (Fonte *et al.* 2016) in both vertebrates and invertebrates by hydrolysing acetylcholine into choline and acetic acid at the cholinergic synapses and neuromuscular junctions (Peña-Llopis *et al.* 2003). It is used as a measure of neurotoxicity following exposure to antibiotics. The available studies prove that, antibiotics reduced AChE activity after exposure to dietary therapeutic ENRO dose (Wang *et al.* 2009) and various doses of bath at sublethal (Li *et al.* 2012), toxicological (Liu *et al.* 2014a; Rodrigues *et al.* 2018b) and both environmental relevant and toxicological levels (Liu *et al.* 2014c), due to their ability to inhibit acetylcholine release on the presynaptic site (Rhee *et al.* 2013; Liu *et al.* 2014a), suppress acetylcholine response postsynaptically and inhibit competitively calcium ions at a common receptor site on the nerve ending membrane (Chow *et al.* 2004). Such inhibitions may cause neuronal and muscle injury, which interrupt the nervous system and ultimately may lead to mortality of cultured fish, affecting aquaculture production (Li *et al.* 2012; Rhee *et al.* 2013; Liu *et al.* 2014a). This might be one of the reasons for the observed body malformation and increased mortality in fish treated with antibiotics.

Notably, antibiotics also increased neurotoxicity after diet and bath exposure using ENRO and CPF at both therapeutic and environmental relevant and toxicological levels in *O. niloticus* brain tissue (Andrieu *et al.* 2015) and roxithromycin at toxicological doses in *C. auratus* (Liu *et al.* 2014b), both studies indicated dose- and time-dependent effects (Liu *et al.* 2014b; Andrieu *et al.* 2015), and the bath treatment caused severe effects than dietary (Andrieu *et al.* 2015). Moreover, exposure to cephalosporins parent antibiotics and metabolites at toxicological concentrations enriched several genes related to neurobehavioral in *D. rerio*, indicating neurotoxicity (Han *et al.* 2018). Several reasons account for increased neurotoxicity in fish after



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antibiotics treatment, including; i) antibiotics act as negative allosteric modulators, which block or change the shape of the orthosteric or allosteric binding sites of ACh receptors, releasing most of the ACh in the synaptic cleft to bind to ChE, thus its activity increases; and ii) antibiotics act as cholinergic agonists or modify the gene coding for choline acetyl-transferase, leading to an increase in ACh production, which is further hydrolysed by ChE resulting in elevated AChE activity (Andrieu *et al.* 2015). Consequently, antibiotics used in fish cause neurotoxicity, affecting the parasympathetic nervous system and ultimately may lead to morbidity of fish exposed to both dietary and bath treatments, as evidenced by high fish mortality reported previously in this review. The neurotoxicity might also be sustained due to compromised immunity and haematological parameters.

In general, antibiotics exposure in cultured fish cause neurotoxicity depending on dose, time and exposure methods. The bath exposure causes higher neurotoxicity compared to dietary treatments due to their higher bioavailability, easier digestibility and assimilation rates (Limbu *et al.* 2018). Nevertheless, AChE/ChE activity as a measure of neurotoxicity induced by antibiotics depends on time such that, the enzymatic activity returns to basal levels after certain period (Andrieu *et al.* 2015). Accordingly, exposure of several fish species to various doses of environmental relevant and toxicological bath antibiotics (Nunes *et al.* 2015; Fonte *et al.* 2016; Rodrigues *et al.* 2018a; Rodrigues *et al.* 2018b; Rodrigues *et al.* 2018c; Almeida *et al.* 2019) did not cause any significant changes in AChE, suggesting time-dependent reversible effects (Andrieu *et al.* 2015). These results suggest careful consideration of time when AChE/ChE activity is used as a measure of neurotoxicity after antibiotics exposure. Therefore, future studies should assess the neurotoxicity caused by antibiotics in cultured fish by using other neurotoxic agents such beta amyloid and glutamate. Furthermore, the role of microbiota in mediating neurodevelopmental toxicity of antibiotics in fish (Catron *et al.* 2019) should also be explored.

## Genotoxicity and apoptosis

The use of antibiotics in cultured fish produces excessive ROS, which directly cause DNA chain breakage and abnormalities, subsequently disrupting the function of DNA repair proteins. Indeed, an appreciable wealth of knowledge exists on the effects of antibiotics on genotoxicity, both for *in vivo* and *in vitro* studies in cultured fish. Notably, for *in vivo* studies, antibiotics induced genotoxicity after exposure to various environmental relevant (Rodrigues *et al.* 2016; Rodrigues *et al.* 2018a), toxicological (Isidori *et al.* 2005; Rocco *et al.* 2012; Yin *et al.* 2014; Wang *et al.* 2016; Załęska-Radziwiłł *et al.* 2016; Rodrigues *et al.* 2018b; Almeida *et al.* 2019; Marques *et al.* 2019), curative (Jerbi *et al.* 2011) doses of bath and *in vitro* (Papis *et al.* 2011; Botelho *et al.* 2015; Załęska-Radziwiłł *et al.* 2016), with some antibiotics indicating more effects than others (Botelho *et al.* 2015). Antibiotics induce genotoxicity in various cultured fish body due to their; i) ability to generate ROS (Papis *et al.* 2011; Rodrigues *et al.* 2016) following oxidation in the presence of transition metal ions (Rodrigues *et al.* 2017a); and ii) strong binding affinity to DNA (Rodrigues *et al.* 2017a), thereby forming antibiotics–DNA binary complexes, which modify the secondary structure of the native DNA strand. The consequences of the two mechanisms maybe DNA strand breakage, base and deoxyribose modifications, DNA cross-links or inhibition of enzymes involved in DNA repair (Rodrigues *et al.* 2016; Rodrigues *et al.* 2017a). Evidently, therapeutic OTC and SMZ diets and their environmental relevant exposure in *O. niloticus* caused DNA damage due to oxidative stress (Limbu *et al.* 2018). Furthermore, exposure to toxicological levels of TC in *D. rerio* increased ROS production, which elevated apoptosis (Zhang *et al.* 2015), similar to *O. niloticus* fed on OTC diet (Limbu *et al.* 2019). These studies demonstrate that, the excessive ROS induced by antibiotics in the previous sections is responsible for genotoxicity and apoptosis in various fish species.

Generally speaking, antibiotics used in cultured fish cause chromosome breakage and/or chromosome abnormalities, which induce genotoxicity and apoptosis. These effects are deleterious because they affect genetic materials directly, which influence fish integrity and may lead to mutations with devastating effects on the overall aquaculture production.

## Detoxification and biotransformation responses

Cytochrome P450 (CYPs), glutathione-s-transferase (GST), permeability glycoprotein (P-gp), metallothionein (MT) and heat shock proteins (HSPs) are useful molecular and/or cellular biomarkers for assessing toxicity induced by pollutants in aquatic organisms (He *et al.* 2011b), due to their ability to biotransform and detoxify xenobiotics in living cells. A wealth of knowledge has been generated over time on the effects of antibiotics on detoxification and biotransformation responses. Consequently, it has been established that, several doses of antibiotics applied as excessive dietary doses (Hu *et al.* 2012; Nakano *et al.* 2018), bath at environmental relevant levels (Liang *et al.* 2015) and injection (Vaccaro *et al.* 2003; Hu *et al.* 2012; Smith *et al.* 2012) for *in vivo* and *in vitro* exposure (Hu *et al.* 2012; Smith *et al.* 2012) inhibited detoxification and biotransformation responses in the liver (Vaccaro *et al.* 2003; Hu *et al.* 2012; Liang *et al.* 2015; Nakano *et al.*


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2018) and muscles and stomach (Nakano *et al.* 2018) in a dose and time (Hu *et al.* 2012), antibiotic type (Smith *et al.* 2012) and gender (Liang *et al.* 2015) specific differences. Such suppression indicates limited ability to counteract excessive oxidative stress caused by antibiotics, which may sustain their toxicity and thus damage fish tissues (Smith *et al.* 2012; Liang *et al.* 2015; Nakano *et al.* 2018). Apparently, we hypothesize that, the inhibited detoxification and biotransformation responses contributed to the observed body damage, immunity suppression, haematological parameters, histopathological changes, neurotoxicity, genotoxicity and apoptosis in the previous sections of this review.

On the other hand, antibiotics also increased detoxification and biotransformation responses, after treatment with dietary doses at therapeutic (Topic Popovic *et al.* 2012; Limbu *et al.* 2018; Limbu *et al.* 2019) and bath at environmental relevant (Barros-Becker *et al.* 2012; Madureira *et al.* 2012; Limbu *et al.* 2018) and toxicological (Barros-Becker *et al.* 2012; Liang *et al.* 2015) levels in a time and species (Topic Popovic *et al.* 2012), gender (Liang *et al.* 2015) specific differences and joint effects (Madureira *et al.* 2011; Madureira *et al.* 2012), indicating excessive oxidative stress (Madureira *et al.* 2011; Madureira *et al.* 2012; Limbu *et al.* 2018; Limbu *et al.* 2019), due to the failure of detoxification response. Essentially, both reduced and increased detoxification and biotransformation responses after antibiotics exposure in cultured fish indicate toxic stress. The induced detoxification toxicity is occasionally similar for mixed and single antibiotics (Qiu *et al.* 2020).

Overall, treating cultured fish with dietary, bath or injection decrease or increase detoxification and biotransformation responses indicating increased stress in fish body, which can damage tissues. Future studies should relate the detoxification response to nutritional metabolism, innate immunity and fish health.

## Potential human health risk via fish consumption

The consumption of fish treated with antibiotics has raised public health concern across the world. However, studies reporting direct human health risk through consumption of antibiotics contaminated fish are currently limited (Limbu *et al.* 2018; Limbu 2020; Limbu *et al.* 2020). To safeguard consumers from risk caused by antibiotics, different countries and agencies set the maximum residue limits (MRLs) of various antibiotics in the fish muscles as 50 μg $kg^{-1}$ for TMP (Romero González *et al.* 2010; Phu *et al.* 2015; Rosa *et al.* 2019) and amoxicillin (Phu *et al.* 2019), 100 μg $kg^{-1}$ for OTC (EMEA 1995; Romero González *et al.* 2010; Elia *et al.* 2014; Rosa *et al.* 2019), SMZ (Phu *et al.* 2015), sulfamethazine (Nunes *et al.* 2018), sulfadiazine (Romero González *et al.* 2010; Rosa *et al.* 2019), OA (EMEA 2005; Rosa *et al.* 2019), ENRO and CPF (EMEA 2002c; Xu *et al.* 2006) and danofloxacin (EMEA 2002b; European Union (EU) 2010; Vardali *et al.* 2017). For other antibiotics, the MRLs are 200 μg $kg^{-1}$ for ERY (EMEA 2002d; Vendrell *et al.* 2012), 300 μg $kg^{-1}$ for difloxacin (EMEA 2002a), 600 μg $kg^{-1}$ for FMQ (Romero González *et al.* 2010; Rosa *et al.* 2019) and 1000 μg $kg^{-1}$ for FFC (EMEA 2000; Gaikowski *et al.* 2015). Exceptionally, the U.S. Food and Drug Administration (U.S. FDA) set the MRL for OTC as 2000 μg $kg^{-1}$ (U.S. EPA 2017).

Based on this information, we analysed the residues of antibiotics in fish muscles as an edible tissue obtained in the current literature to understand the possible human health risk resulting from direct consumption of fish treated with antibiotics used in global aquaculture (Table 8). We found that, the residues of most studied antibiotics detected in muscles from various fish species during different sampling periods were higher than MRLs established for most antibiotics such as OTC (Björklund & Bylund 1990; Malvisi *et al.* 1996; Namdari *et al.* 1996; Namdari *et al.* 1998; Meinertz *et al.* 2001; Bebak-Williams *et al.* 2002; Rigos *et al.* 2002; Bernardy *et al.* 2003; Chen *et al.* 2004; Zhang & Li 2007; Romero González *et al.* 2010; Paschoal *et al.* 2012; Elia *et al.* 2014; Limbu *et al.* 2018; Rosa *et al.* 2019; Vorbach *et al.* 2019), FFC (Wrzesinski *et al.* 2006; Feng *et al.* 2008; Lim *et al.* 2010; Di Salvo *et al.* 2013; Meinertz *et al.* 2014; Gaikowski *et al.* 2015; Marques *et al.* 2018), TMP (Phu *et al.* 2015; Zonaras *et al.* 2016; Rosa *et al.* 2019), SMZ (Phu *et al.* 2015; Limbu *et al.* 2018), sulfamethazine (Nunes *et al.* 2018), sulfadiazine (Romero González *et al.* 2010; Zonaras *et al.* 2016; Rosa *et al.* 2019), FMQ (Tyrpenou *et al.* 2003), ENRO (Intorre *et al.* 2000; Xu *et al.* 2006), CPF (Xu *et al.* 2006), ERY (Vendrell *et al.* 2012), danofloxacin (Vardali *et al.* 2017), amoxicillin (Phu *et al.* 2019) and difloxacin (Ding *et al.* 2006). Probably, the inhibited detoxification and biotransformation responses facilitated the deposition of antibiotics in fish muscles, resulting in higher MRLs. The higher MRLs of antibiotics residues in fish muscles indicate the existence of human health risk upon consumption without considering withdrawal periods (Table 8). In most of these studies, the antibiotics residues were lower than MRLs after appropriate withdrawal periods, except for ENRO (Lucchetti *et al.* 2004), TMP (Romero González *et al.* 2010; Rosa *et al.* 2019) and OTC (Bernardy *et al.* 2003; Elia *et al.* 2014; Rosa *et al.* 2019), implying human health risk even after withdrawal periods. In fact, higher MRLs have been reported in seafood products by the rapid alert system for food and feed (RASFF) of the European Commission from eight countries as detailed by Barani and Fallah (2015) (Table S1). In 2017, the RAFF also reported four cases of OTC residues above MRLs from Chile (RAFF 2018). These



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**Table 8** Residues of antibiotics in muscles of cultured fish

| Antibiotic | Dosage, exposure method and duration | Fish species and size | Residue (μg/kg) and time detected | Reference |
|---|---|---|---|---|
| Oxytetracycline | Dietary; 88.2 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Oncorhynchus kisutch*; 13–62 g | 932; 1 day after last treatment | Meinertz *et al.* (2001) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$: 42 days | *Salmo gairdneri*; 246 ± 78 g at 16 °C | Below detection; 32 days after last treatment. | Björklund and Bylund (1990) |
| | | *Salmo gairdneri*; 246 ± 78 g at 10 °C | Below detection; 32 days after last treatment | |
| | | *Salmo gairdneri*; 246 ± 78 g at 5 °C | 300; 40 days after last treatment | |
| Oxytetracycline | Dietary; 75, 150, 300 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Cyprinus carpio*: 108.3 ± 27.6 g | 295; after 10 days of treatment | Elia *et al.* (2014) |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$: 14 days | *Sparus aurata*; 50-70 g | 1230 ± 720; after 6 days of treatment | Malvisi *et al.* (1996) |
| | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$: 14 days | *Dicentrarchus labrax;* 80-100 g | 20; after 6 days of treatment | |
| Oxytetracycline | Dietary; 82.8 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sander vitreus*; 150 g | 3120 ± 2790; 1 h | Chen *et al.* (2004) |
| | | *Paralichthys dentatus*; 350 g | 950; 1 h after 10 days of treatment | |
| | | ♀ *Morone chrysops* × ♂ *M. saxatilis*; 188 g | 1500 ± 250; 1 h after 10 days of treatment | |
| | | *Oreochromis niloticus*; 450 g | 1230 ± 470; 1 h after 10 days of treatment | |
| Oxytetracycline | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata;* 150 – 200 g at 14 °C | 304; 1 day after treatment | Romero González *et al.* (2010) |
| | | *Sparus aurata;* 150 – 200 g at 19.5 °C | 109; 1 day after treatment | |
| Oxytetracycline | Dietary; 66 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Oncorhynchus mykiss*; 562 g | 1820: 10 days of treatment | Bebak-Williams *et al.* (2002) |
| Oxytetracycline | Intravascular; 40 mg $kg^{-1}$ body weight $day^{-1}$ | *Dicentrarchus labrax;* 110 g at 13.5 °C | 2000 ± 700; 1 h postinjection | Rigos *et al.* (2002) |
| | | *Dicentrarchus labrax;* 110 g at 22 °C | 13500 ± 630; 1 h postinjection | |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$; 21 days | *Oncorhynchus tshawytscha*; 55 g at 9 °C | 4480 ± 1630; 1 day postdosing | Namdari *et al.* (1996) |
| | | *Oncorhynchus tshawytscha*; 55 g at 15 °C | 4350 ± 1390; 1 day postdosing | |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 42 days | *Oncorhynchus kisutch*; 20 g at 10 °C | 950 ± 440; $7^{th}$ day of medication | |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ body weight $day^{-1}$; 5 days | *Orechromis niloticus*; 7-day-old | 128; 1 day after medication | Paschoal *et al.* (2012) |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Oncorhynchus tshawytscha*; 400 g at 9 °C | 780 ± 650; $10^{th}$ day of medication | Namdari *et al.* (1998) |
| | | *Oncorhynchus tshawytscha*; 400 g at 12 °C | 1040 ± 480; $10^{th}$ day of medication | |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Salmo salar*; 2000 g | 1210 ± 400; $10^{th}$ day of medication | |
| Oxytetracycline | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$; 7 days | *Ctenopharyngodon idellus*; 100 ± 6 g | 5500 ± 280; 1 day postdosing | Zhang and Li (2007) |
| Oxytetracycline | Dietary; 82.7 mg $kg^{-1}$ body weight $day^{-1}$; 10 days | *Esox lucius*; 115 g | 319; 2 days of depuration | Bernardy *et al.* (2003) |
| | | *Stizostedion vitreum*; 59 g | 549; 2 days of depuration | |
| Oxytetracycline | Dietary; 75 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Dicentrarchus labrax;* 50.1 ± 0.9 g | 5683.17; $7^{th}$ day of treatment | Rosa *et al.* (2019) |
| Oxytetracycline | Dietary; 80 mg $kg^{-1}$ body weight $day^{-1}$: 84 days | *Oreochromis niloticus*; 27.73 ± 0.81 g | 118319.40 ± 5685.69; 84 days of treatment | Limbu *et al.* (2018) |


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**Table 8** (continued)

| Antibiotic | Dosage, exposure method and duration | Fish species and size | Residue (μg/kg) and time detected | Reference |
|---|---|---|---|---|
| Oxytetracycline | Exposure; 400 mg $L^{-1}$: 12 h | *Devario aequipinnatus*; 2.5 ± 0.6 g | 1467 ± 469.66; 12 h of treatment | Vorbach *et al.* (2019) |
| Florfenicol | Dietary; 15 mg $kg^{-1}$ body weight $day^{-1}$: 12 days | *Oreochromis niloticus*; 507 ± 86 g | 1050; 1 day postdosing | Gaikowski *et al.* (2010) |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Oncorhynchus mykiss*; 220 ± 30 g | 4082 ± 1261; 6 h post-treatment | Di Salvo *et al.* (2013) |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata*; 150 ± 20 g | 1578 ± 434; 1 day post-treatment | |
| Florfenicol | Dietary; 9.3 mg $kg^{-1}$ body weight $day^{-1}$: 12 days | *Ictalurus punctatus;* 900 g | 5378 ± 7014; 1 day after dosing | Wrzesinski *et al.* (2006) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Oreochromis spp.* | 13770; 1 h postdosing | Gaikowski *et al.* (2015) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Oncorhynchus mykiss*; 126–617 g | 1158; 12 h post-treatment | Meinertz *et al.* (2014) |
| Florfenicol | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$: 3 days | *Paralichthys olivaceus*, 380.4 ± 20.8 g | 3060 ± 780; 1 day after last dose | Lim *et al.* (2010) |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$: 12 h | *Oreochromis niloticus* × *O. aureus)*; 115 g in freshwater | 4590; 12 h after administration | Feng *et al.* (2008) |
| | | *Oreochromis niloticus* × *O. aureus)*; 115 g in seawater | 3600; 12 h after administration | |
| Florfenicol | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Piaractus mesopotamicus*; 724 g | 878; 1 day post-treatment | Marques *et al.* (2018) |
| Trimethoprim | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata;* 150 – 200 g at 14 °C | 22; 1 day after the last administration | Romero González *et al.* (2010) |
| | | *Sparus aurata;* 150 – 200 g at 19.5 °C | 32; 1 day after the last administration | |
| Trimethoprim | Dietary; 113 ± 11.0 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Pangasianodon hypophthalmus*; 40.3 ± 7.2 g | 92.5 ± 83.7; $5^{th}$ day during medication | Phu *et al.* (2015) |
| Trimethoprim | Dietary; 113 ± 11.0 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Oreochromis mossambicus* × *O. niloticus*; 10.7 ± 4.5 g | 136 ± 52; $5^{th}$ day during medication | |
| Trimethoprim | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Sparus aurata*; 230 ± 45 g fed fish oil diet | 697.3 ± 372.0; 1 day during medication | Zonaras *et al.* (2016) |
| | | *Sparus aurata*; 230 ± 45 g fed plant oil diet | 269.4 ± 156.6; 1 day during medication | |
| Trimethoprim | Dietary; 44 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Dicentrarchus labrax;* 50.1 ± 0.9 g | 11458.7; $7^{th}$ day of treatment | Rosa *et al.* (2019) |
| Sulfamethoxazole | Dietary; 674 ± 10.1 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Oreochromis mossambicus* × *O. niloticus*; 10.7 ± 4.5 g | 603 ± 255; $5^{th}$ day during medication | Phu *et al.* (2015) |
| | | *Pangasianodon hypophthalmus*; 40.3 ± 7.2 g | 577 ± 179; $5^{th}$ day during medication | |
| Sulfamethoxazole | Dietary; 100 mg $kg^{-1}$ body weight $day^{-1}$: 84 days | *Oreochromis niloticus*; 27.73 ± 0.81 g | 19722 ± 3933; 84 days of treatment | Limbu *et al.* (2018) |
| Sulfamethazine | Dietary; 422 mg $kg^{-1}$ body weight $day^{-1}$: 11 days | *Oreochromis niloticus*; 30 ± 5 g | 1600; 11 days after treatment | Nunes *et al.* (2018) |
| Sulfadiazine | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata;* 150 – 200 g at 14 °C | 268; 1 day after the last administration | Romero González *et al.* (2010) |
| | | *Sparus aurata;* 150 – 200 g at 19.5 °C | 249; 1 day after the last administration | |
| Sulfadiazine | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Sparus aurata*; 230 ± 45 g fed fish oil diet | 1794.0 ± 863.4; 1 day during medication | Zonaras *et al.* (2016) |
| | | *Sparus aurata*; 230 ± 45 g fed plant oil diet | 954.1 ± 496.8; 1 day during medication | |


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**Table 8** (continued)

| Antibiotic | Dosage, exposure method and duration | Fish species and size | Residue (μg/kg) and time detected | Reference |
|---|---|---|---|---|
| Sulfadiazine | Dietary; 220 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Dicentrarchus labrax;* 50.1 ± 0.9 g | 15351.65; 7$^{th}$ day of treatment | Rosa *et al.* (2019) |
| Flumequine | Dietary; 35 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Sparus aurata*; 236.68 ± 36.53 g at 18 °C | 2394; 1 day after the last dosing | Tyrpenou *et al.* (2003) |
| | | *Sparus aurata*; 307 ± 29.55 g at 24 °C | 1225; 1 day after the last dosing | |
| Flumequine | Dietary; 12 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Sparus aurata*; 60-80 g | 68.7 ± 49.6; 1 day after treatment | Malvisi *et al.* (1997) |
| Flumequine | Dietary; 12 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Dicentrarchus labrax;* 50.1 ± 0.9 g | 386.63; 7$^{th}$ day of treatment | Rosa *et al.* (2019) |
| Flumequine | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata;* 150 – 200 g at 14 °C | 55; 1 day after the last administration | Romero González *et al.* (2010) |
| | | *Sparus aurata;* 150 – 200 g at 19.5 °C | 11; 1 day after the last administration | |
| Oxolinic acid | Dietary; 30 mg $kg^{-1}$ body weight $day^{-1}$: 10 days | *Sparus aurata;* 150 – 200 g at 14 °C | 62; 1 day after the last administration | Romero González *et al.* (2010) |
| | | *Sparus aurata;* 150 – 200 g at 19.5 °C | 8; 1 day after the last administration | |
| Oxolinic acid | Dietary; 12 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Dicentrarchus labrax;* 50.1 ± 0.9 g | 1680.4; 7$^{th}$ day of treatment | Rosa *et al.* (2019) |
| Enrofloxacin | Dietary; 50 mg $kg^{-1}$ body weight $day^{-1}$: 7 days | *Oreochromis niloticus*; 160-240 g | 3610; 1 h postdosing | Xu *et al.* (2006) |
| Ciprofloxacin | Metabolite | | 220; 1 h postdosing | |
| Enrofloxacin | Dietary; 5 mg $kg^{-1}$ body weight $day^{-1}$: 96 h | *Dicentrarchus labrax;* 200–300 g | 1130 ± 280; 10 h of treatment | Intorre *et al.* (2000) |
| Enrofloxacin | Dietary; 10 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Oncorhynchus mykiss*; 250 g | 15450 ± 679; 3 h after treatment | Lucchetti *et al.* (2004) |
| Ciprofloxacin | Metabolite | | 510 ± 150; 3 h after treatment | |
| Erythromycin | Dietary; 52 mg $kg^{-1}$ body weight $day^{-1}$: 8 days | *Oncorhynchus mykiss*; 152 ± 4 g | 1173.83; 2 days post-treatment | Vendrell *et al.* (2012) |
| Danofloxacin | Dietary; 12 mg $kg^{-1}$ body weight $day^{-1}$: 5 days | *Dicentrarchus labrax*; 191.60 ± 15.27 g | 712.0 ± 166.5; 1 day after last dosing | Vardali *et al.* (2017) |
| Difloxacin | Dietary; 20 mg $kg^{-1}$ body weight $day^{-1}$: 3 days | *Carassius auratus*; 250 ± 30 g at 10 °C | 32670 ± 2550; 1 day after the last administration | Ding *et al.* (2006) |
| | | *Carassius auratus*; 250 ± 30 g at 22 °C | 14460 ± 5630; 1 day after the last administration | |

data suggest human health risk from direct consumption of fish and other seafood treated with antibiotics.

In practise, these results signify high human health risk after direct consumption of fish treated with antibiotics because of several reasons. First, fish farmers mostly violate withdrawal periods (Olatoye & Basiru 2013) and apply antibiotics on daily basis and in absence of diseases (Defoirdt *et al.* 2011; Pham *et al.* 2015; Phu *et al.* 2015) to sick, healthy and carrier fish individuals (Cabello *et al.* 2013; Cabello *et al.* 2016). Second, withdrawal periods for antibiotics vary among cultured fish species and are dependent on water quality parameters such as temperature (Björklund & Bylund 1990) and salinity (Rairat *et al.* 2020). Furthermore, withdrawal periods are complicated by existence of antibiotics metabolites, which differ in toxicity and concentrations than their parent compounds (Xu *et al.* 2006; Lim *et al.* 2010; Marques *et al.* 2018). As such, analysing human health risk of the parent antibiotic by considering withdrawal periods may underestimate the risk. Indeed, direct human health risk has been reported in children following consumption of *O. niloticus* treated with dietary OTC after chronic exposure without considering withdrawal period (Limbu *et al.* 2018). However, the human health risk caused by antibiotics is dependent on exposure period. Clearly, feeding *O. niloticus* with OTC diet for 35 days did not cause any human health risk (Limbu *et al.* 2020). Third, human health risk obtained in the reported studies might be high due to consumption of antibiotic residues from multiple sources such as wild fish (Björklund *et al.* 1990; He *et al.* 2011b; Gao *et al.* 2012; Zhao *et al.*


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2015; Ben *et al.* 2019), other cultured fish from farms (Ansari *et al.* 2014; Barani & Fallah 2015; Fadaeifard *et al.* 2015; Monteiro *et al.* 2015; He *et al.* 2016; Chen *et al.* 2018) and markets (Olatoye & Basiru 2013; Adel *et al.* 2017), other seafood such as shrimps (Sun *et al.* 2016; Ben *et al.* 2019) and crabs (Song *et al.* 2017). In addition, antibiotics residues are also obtained from consumption of beef, pork and dairy products (Ji *et al.* 2010; Vragović *et al.* 2012), contaminated vegetables (Robles Jimenez *et al.* 2018), drinking water (Cui *et al.* 2018) and milk (Vragović *et al.* 2012; Wang *et al.* 2017) and existence of resistance genes in fish (Shah *et al.* 2012; Shah *et al.* 2014; Xu *et al.* 2017), other food-producing animals (Van *et al.* 2020; Liu *et al.* 2020a) and resistance bacteria (Van *et al.* 2020). In some of these studies, higher levels of antibiotics than MRLs were also reported, indicating human health risk (Olatoye & Basiru 2013; Ansari *et al.* 2014; Monteiro *et al.* 2015; Adel *et al.* 2017). Lastly, the human health risk resulting from consumption of fish may also be high because antibiotics residues in different foods are not eliminated by common food processing procedures such as cooking and freezing (O'Brien *et al.* 1981), ordinary cooking (Gajda *et al.* 2017) and frying or baking (Bacanlı & Başaran 2019).

Nevertheless, a few studies reported antibiotics residues lower than MRLs for FFC in *O. niloticus* (Gaikowski *et al.* 2010), TMP and OTC in *S. aurata* (Romero González *et al.* 2010), OTC in *S. gairdneri* (Björklund & Bylund 1990) and most studies conducted using FMQ in various fish species (Malvisi *et al.* 1997; Romero González *et al.* 2010; Rosa *et al.* 2019) (Table 8). The differences in the results obtained are caused by variations in antibiotics doses, fish species and size, duration of the experiments and sampling periods (Table 8). Notably, depletion of antibiotics is higher for freshwater than marine water species (Chen *et al.* 2004; Feng *et al.* 2008) and is increased by higher water temperature (Björklund & Bylund 1990; Namdari *et al.* 1996; Rigos *et al.* 2002; Tyrpenou *et al.* 2003; Ding *et al.* 2006; Romero González *et al.* 2010). The variations in the results might also be caused by additive effects of antibiotics used in aquaculture and those existing in the water used for fish culture, such as river water or seawater.

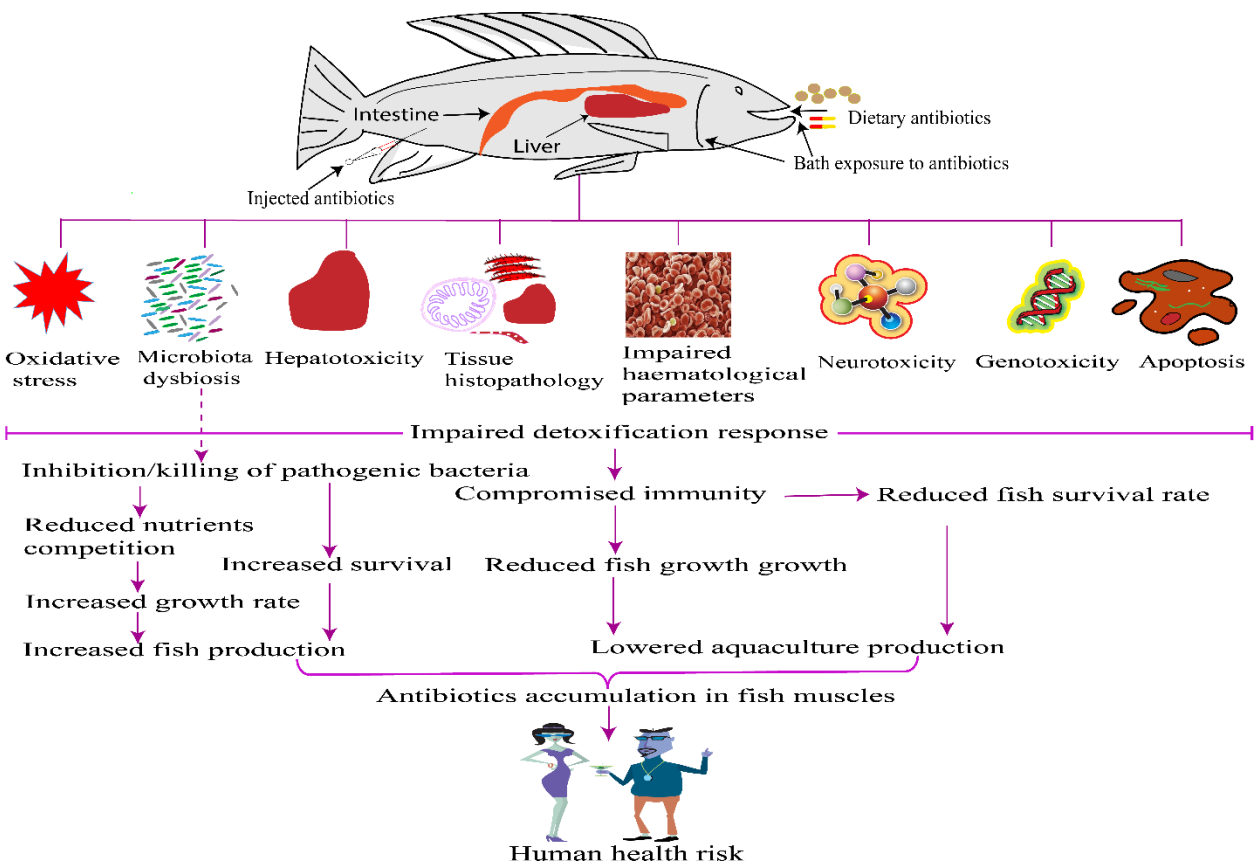



**Figure 2** A possible generalized summary of the systemic effects of antibiotics in cultured fish.




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Taken together, the higher MRLs and the results from Limbu *et al.* (2018) should provide an alarm that, direct consumption of cultured fish treated with antibiotics holds potential public health risk. The depletion of antibiotics in different muscles of cultured fish is a complex process determined by species and their size, antibiotic types and duration of exposure, water quality parameters and dietary composition. Analysing human health risk from consumption of fish treated with antibiotics by considering withdrawal periods might underestimate the risk because of its variations among fish species, metabolites and parent antibiotics. More studies estimating direct human health risk from consumption of fish treated with antibiotics, which consider both parent antibiotics and their metabolites are required and joint risk from multiple sources are needed in the future.

## Concluding remarks and further perspectives

The OTC dominates the current antibiotics used in aquaculture globally. Antibiotics produce several systemic effects on cultured fish based on their types, doses, exposure method and duration, and fish species and growth stage (Fig. 2). Most of antibiotics studied induce oxidative stress through generation of excessive ROS, which cause hepatotoxicity and several histopathological effects. Antibiotics also cause microbiota dysbiosis due to their ability to interfere with normal microbiota, which subsequently affect nutrients metabolism and immunity. Moreover, antibiotics affect haematological parameters due to their toxic effects, which subsequently impair detoxification response, sustaining the toxicity of antibiotics by inducing neurotoxicity, genotoxicity and apoptosis, which in turn compromise the fish immunity response. The compromised immunity causes body damage, which affect the survival rate of cultured fish. Lastly, antibiotics affect growth performance of the cultured fish through increased oxidative stress, body damage and impaired nutrients metabolism and feed utilization efficiency. All these finally affect the overall aquaculture production. Despite this, some antibiotics increase growth and survival rates of fish by reducing pathogenic bacteria abundance, which decreases nutrients competition between the host and bacteria, consequently increases accumulation of antibiotics in fish muscle (Fig. 2).

The consumption of fish treated with antibiotics poses human health risk due to the higher MRLs and hazard risk quotient, which provide an alarm to the general public. Possibly, the human health risk is underestimated by considering withdrawal periods because of violation by fish farmers, variations in withdrawal periods for the same antibiotic among fish species and toxicity differences between metabolites and parent antibiotics coupled with consumption of multiple sources of contaminated foods. Thus, the clinical use of antibiotics on food-fish species should be considered with a caution and, if used, monitoring should be performed.

Overall, much research remains to be performed in order to understand precisely the systemic effects of antibiotics in fish. First, research should be directed towards studying holistic toxicological effects of antibiotics on host fish such as body function, physiology, metabolism and reproduction effects in fish for mixtures and their metabolites rather than single antibiotic owing to their synergistic or antagonist interactions (Zhang *et al.* 2016). Second, the variations in effects among antibiotics and fish species should also be elucidated by exploring the influence of water quality parameters in the culture media on antibiotics toxicity. Third, future studies should be carried out on the possibility of replacing antibiotics by using other agents capable of inhibiting or killing bacteria. Fourth, research on mechanisms to restore the antibiotic-induced toxicity in fish body are required because methods for replacing antibiotics are unavailable or still under research phase, while antibiotics are in use. Lastly, human health risk should be assessed directly from multiple consumption of both parent and their metabolites and joint antibiotics.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Occurrence of antibiotic residues higher than MRLs in seafood products reported by RASFF of the European Commission.


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