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Invited Review Article

Unravelling the fate of florfenicol and its transformation products in the aquatic ecosystem: A review

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Abstract

Aquaculture has emerged as a sustainable solution to meet global protein demand and support livelihoods. Antibiotics, notably florfenicol (FFC), have transformed aquaculture, enhancing disease management and productivity. However, FFC's widespread use has led to concerns about antibiotic resistance, ecological disruption and human health risks. This brief review delves into the FFC's impact on aquatic microflora, its transformation products (TPs) and the fate of TPs in the water and aquatic sediments. Florfenicol's administration triggered microbial community shifts, promoting antibiotic-resistant genes and altering aquatic ecosystems. The formation of diverse TPs further raised concerns due to their potential toxicity and persistence. Critically evaluating FFC's fate is essential to establish regulatory measures and comprehending its ecological implications. This review further underscored the urgent need for responsible FFC use in food-animal production systems and highlights knowledge gaps for future research endeavours.

Keywords: Amphenicol, Aquaculture, Aquatic environment, Aquatic microflora, Florfenicol degradation

Highlights

- Elucidating the impact of florfenicol transformation in aquatic environments exploring florfenicol's influence on aquatic microbial community dynamics. Proposed safe usage guidelines for florfenicol in food-animal production systems.
- Unveiling a tentative degradation pathway for florfenicol in aquatic environments.
- Calling for future research on florfenicol's residues and transformation products in aquaculture.

INTRODUCTION

Aquaculture has been playing a vital role in meeting the increasing global demand for animal protein and supporting the livelihoods of millions of people worldwide (FAO, 2022). As traditional capture fishing faced challenges through overfishing and depleted fish stocks, aquaculture has emerged as a sustainable and efficient solution to supplement protein production and ensure food security (FAO, 2022). Aquaculture production has grown remarkably over the years, utilizing diverse aquatic resources and cultivation methods to cater to different market demands and environmental considerations. In 2020 alone, global aquaculture production reached 122.6 million tonnes with a total value of USD 281.5 billion (FAO, 2022). With the ever-growing demand for protein due to population growth and changing dietary preferences, the aquaculture sector is projected to continue expanding. As the industry is facing increased demand, it must address challenges related to sustainability, environmental impact, disease management and social and economic issues for long-term viability. The advent of antibiotics and intensification in culture activities has brought about a revolutionary transformation in aquaculture, significantly impacting the industry's productivity and sustainability (FAO, 2021). Antibiotics have played a crucial role in the development of intensive aquaculture systems, allowing for higher stocking densities and better biosecurity practices. By administering antibiotics to fish and aquatic organisms, farmers can control and treat various diseases, enhancing overall survival rates and promoting healthier growth. This has led to increased yields and improved economic viability for aquaculture operations worldwide (FAO, 2021). Despite the significant benefits, it is essential to use antibiotics responsibly in aquaculture to avoid potential risks, such as the development of antibiotic resistance and the dissemination of antibiotic residues in the environment and aquatic organisms. The widespread usage and overuse of antibiotics have had

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profound and detrimental effects on the aquatic environment. One of the primary concerns is the emergence and spread of antibiotic-resistant bacteria (ARB) and the disruption of the natural balance of microbial communities, leading to ecological imbalances and potential disruptions in nutrient cycling in aquatic environments (FAO, 2021).

The transformation or transformed products (TPs) of antibiotics, which are formed through various physical, chemical and biological processes in the aquatic environment, can also have significant effects on aquatic ecosystems (Jia *et al.*, 2022). The TPs can result from the degradation, breakdown, or alteration of antibiotics over time. While some TPs may be less active or even less toxic than the parent antibiotics, others can be equally or more harmful to aquatic organisms and the ecosystem (Jia *et al.*, 2022). In some cases, the TPs can persist in the environment for extended periods, further increasing their potential to cause harm. Moreover, the transformation of antibiotics can lead to the formation of new compounds with unknown properties, making it challenging to assess their ecological and human health impacts (Chen *et al.*, 2018). Consequently, the presence of TPs of antibiotics in the aquatic environment raised concerns about their potential to disrupt microbial communities, contribute to antibiotic resistance, and adversely affect the health of aquatic organisms (Kümmerer, 2009). To safeguard the aquatic environment, it is essential to consider the fate of TPs of antibiotics and conduct thorough assessments of their risks when evaluating the impact of antibiotic usage in aquatic systems. In light of the escalating use of antibiotics and concerns about environmental residues, understanding the depletion and metabolism processes of antibiotics in aquaculture becomes crucial to ensure minimal residue levels and safeguard the health of cultured aquatic products (FAO, 2021). The present review offers comprehensive aspects of florfenicol (FFC) depletion in the aquatic environment including sediment, including its TPs. By providing an integrated outlook, this review aims to shed light on the various TPs of FFC in the aquatic environment, contributing to a better understanding of its fate and potential impacts on aquatic ecosystems.

Antibiotics in aquaculture

The introduction of antibiotics in aquaculture marked a significant milestone in the food industry, providing a valuable tool for disease management and improving the health security of farmed fish and shellfish. One of the first documented instances of the use of antibiotics in aquaculture was the use of sulfonamides to treat brook trout *Salvelinus fontinalis* against furunculosis caused by *Aeromonas salmonicida* (Adenaya *et al.*, 2023). Among the sulfonamides, sulfadimethoxine (potentiated with ormetoprim) via medicated feed has been approved for the control of furunculosis in freshwater-reared salmonids and enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri*. Sulfamerazine fish grade has also been used in aquaculture after its approval in 1967 (USFDA, 2022). Subsequently, oxytetracycline dihydrate (OTC), a broad-spectrum antibiotic was approved for aquacultural use and has since become one of the most widely used antibiotics in the industry (USFDA, 2022). Its success in controlling ulcers caused by *Hemophilus piscium*, furunculosis, bacterial hemorrhagic septicemia caused by *A. hydrophila*, *Pseudomonas* disease, gaffkemia caused by *Aerococcus viridans* and columnaris disease caused by *Flavobacterium columnare* (USFDA, 2022) further bolstered its adoption in aquaculture. As research and development continued, FFC was introduced and approved for aquaculture use in 2015 (USFWS, 2015). This antibiotic has shown superior potential compared to some previous antibiotics, even outperforming its analogue thiamphenicol and has outpaced OTC as the most used antibiotic in aquaculture. Even though it was first permitted for its superior efficacy in controlling mortality caused by enteric septicemia of catfish, columnaris and furunculosis, it was later approved for controlling the mortality caused by streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish (USFDA, 2022). The usage of antibiotics in aquaculture has indeed brought a sense of health security to the industry by enabling effective disease prevention and treatment. A detailed list of approved and authorized or licensed antibiotics in global aquaculture and their corresponding dose and dosage is provided in Table 1.

Florfenicol in aquaculture

Florfenicol (FFC), a fluorinated synthetic analogue of thiamphenicol, demonstrated broad-spectrum bacteriostatic activity against a wide range of microorganisms by inhibiting bacterial protein biosynthesis through reversible binding to the peptidyl transferase centre at the 50S ribosomal subunit (Assane *et al.*, 2019). As an approved antibiotic in aquaculture, FFC has been widely employed to treat bacterial infections and ensure animal welfare, surpassing chloramphenicol, thiamphenicol, and even the approved antibiotic OTC in terms of efficacy. Safety trials using Aquaflor, a commercial premix containing FFC, and pure FFC powder have been conducted on various

Table 1. List of approved and authorized or licensed antibiotics in global aquaculture and their corresponding dose and dosage

Antibiotic	Dose	References
Approved		
Oxytetracycline	80 mg/kg fish biomass/day for 10 consecutive days	USFDA, 2022
Florfenicol	10-15 mg/kg fish biomass/day for 10 consecutive days	USFDA, 2022
Sulfadimethoxine + ormetoprim (5:1)	50 mg/kg fish biomass/day for 5 consecutive days	USFDA, 2022
Sulfamerazine	100-200 mg/kg fish biomass/day for 10-20 days	Austin and Austin, 2016
Authorized/Licensed*		
Enrofloxacin	10 mg/kg fish biomass/day for 5 consecutive days	Wang *et al.*, 2016
Oxolinic acid	12 mg/kg fish biomass/day for 7 consecutive days	Giraud *et al.*, 2006
Flumequine	20 mg/kg fish biomass/day for 5 consecutive days (Freshwater) 40 mg/kg fish biomass/ day for 5 consecutive days (Seawater)	Lunestad and Samuelsen, 2008
Erythromycin	50 mg/kg fish biomass/day for 5 consecutive days	Seo *et al.*, 2014

*Authorized and licensed for aquacultural use in China, Vietnam and Korea

aquacultural species (Gaikowski *et al.*, 2003; Basti *et al.*, 2011; Gaikowski *et al.*, 2013; Bardhan *et al.*, 2022a,b; Mallik *et al.*, 2023). FFC is extensively used as a substitute for chloramphenicol in aquaculture and is known for its low negative effects and potent sterilization properties. It has been recommended for treating various fish bacterial diseases (USFDA, 2022). However, the substantial usage of FFC is a concern, with over 10,000 tons used in agriculture and medicine industries in China alone, leading to its presence in aquatic environments through wastewater discharges (Shao *et al.*, 2021). In Chile, FFC is known to be the most commercially used antibiotic to date in salmon farming (Cabello *et al.*, 2023). In this context, the report on the importation of over 493 tons of FFC for veterinary purposes in Chile during 2020 was noteworthy. According to Sernapesca (2021), a substantial 74% of this quantity was designated for employment in aquaculture, in stark contrast to the minimal import of approximately 0.1 tons of chloramphenicol for human usage. The predominant driving force behind antimicrobial resistance (AMR) in the region is the utilization of fenicol compounds within the realm of salmon aquaculture in Chile. While the Chile governmental regulatory bodies, namely Servicio Agricola Ganadero (SAG), reported sales of 344 tons of FFC for aquaculture use, Sernapesca (2021) testified for 365 tons, prompting a call for improvements in regulatory practices. Since FFC residues are quite stable in aquatic environments, they can pose potential risks to non-target organisms and contribute to antibiotic resistance.

Despite their benefits, the use of antibiotics in aquaculture has faced criticism due to concerns surrounding antibiotic resistance, persistent residue presence and consumer safety (Yuan *et al.*, 2023). Despite FFC's effectiveness, concerns have been raised regarding the presence of FFC residues in the aquatic environment, where it transforms into various TPs (Jia *et al.*, 2022) including florfenicol amine (FFA) (Bardhan *et al.*, 2022b; Sumithra *et al.*, 2022). These residues, detected through diverse analytical techniques, contribute to AMR and microbial dysbiosis, potentially compromising the antibiotic's efficacy (Zeng *et al.*, 2019; Zheng *et al.*, 2021). Excessive use of FFC in animal husbandry can lead to the prevalence and abundance of FFC-resistant genes (FRGs) in the environment (Zeng *et al.*, 2019; Zhang *et al.*, 2022). FFC has stable physical and chemical properties, making it resistant to degradation, and its continuous discharge, along with unabsorbed residues, contributed to its persistent presence in the environment. Consequently, varying concentrations of FFC residues have been detected in coastal waters, sediments and aquatic organisms (Marques *et al.*, 2018; Yang *et al.*, 2020; Zhang *et al.*, 2020, 2021). To ensure consumer food safety, maximum residue limits (MRLs) and drug withdrawal periods have been specified for FFC. The European Commission recommended that total residues of FFC and FFA in finfish should not exceed 1000 μg/kg (EU, 2008), while China set the MRL for FFC and its metabolites in fish muscle at the same level (Limbu *et al.*, 2020). However, in practical applications, higher dosages of FFC may be necessary to achieve immediate effects, necessitating effective methods for depleting FFC residues from fish tissues Zhang *et al.* (2023a) revealed that a significant portion, approximately 45–60%, of FFC remains unabsorbed by animals and is subsequently excreted in manure and urine. Moreover, FFC demonstrated stable physicochemical properties, allowing it to persist in aquatic systems under typical conditions of ambient temperature and pH found in natural waters. Alarmingly, FFC has also been found in human urine, highlighting its potential health risks (Zhang *et al.*, 2021). Despite its widespread use in farming worldwide, the negative environmental impacts of FFC, especially on aquatic ecosystems, still present a challenging evaluation. As FFC exhibits limited aqueous degradability, its extensive application in the domains of livestock and aquaculture may pose considerable challenges. Previous inquiries have delved into the fate of FFC within ice-covered reservoirs (Sun *et al.*, 2023), watercourses receiving effluents from wastewater treatment plants (Jara *et al.*, 2021) and controlled aquatic simulations (Sun *et al.*, 2012). This review henceforth focused on elucidating the pathways of formation and the enduring existence of all discernible TPs derived from FFC in aquatic environments. Notably, this assessment marks the foremost exhaustive compilation of all documented TPs of FFC to the present date.

Effect of florfenicol on aquatic microorganisms

Microorganisms play crucial roles in providing essential ecosystem services, such as nutrient recycling, organic matter mineralization and pollution degradation (Näslund *et al.*, 2008). When antibiotics enter aquatic ecosystems, they can induce changes in the microbial community structure, affecting the ecological functions of these ecosystems, including biomass and nutrient production. Antibiotics have the potential to disrupt the equilibrium and structural diversity of microbial communities, leading to long-term or even permanent consequences (Di Cesare *et al.*, 2013). FFC is known to demonstrate a negative impact on the sediment microbial community, which could have cascading effects on other organisms over time (Sun *et al.*, 2012). Consequently, the presence of FFC residues in aquatic sediments may prolong the harmful impact on aquatic microflora, and it is, therefore, crucial to prohibit the misuse of antibiotics in aquatic ecosystems to safeguard their ecological integrity (Näslund *et al.*, 2008). Fish, like all multicellular organisms, host diverse microbial communities. These microbes contribute a vast number of functional genes, exceeding host genes by 100-fold, playing essential roles in nutrient metabolism, host immunity and even cognitive and behavioural modulation (Zhang *et al.*, 2023a). The antibiotic treatment, exemplified by FFC, disrupted these beneficial contributions by altering bacterial community composition, reducing diversity, and exerting selective pressure on resistance-related genes and mutations in the environmental resistome (Zhang *et al.*, 2023a). FFC's unique structure may confer resistance to inactivation by CAT enzymes, further influencing microbial communities in water (Zhang *et al.*, 2023b). Nevertheless, over time, richness and diversity tend to recover. Understanding these antibiotic effects can shed light on the genetic adaptations of aquatic microbial communities (Zhang *et al.*, 2023b).

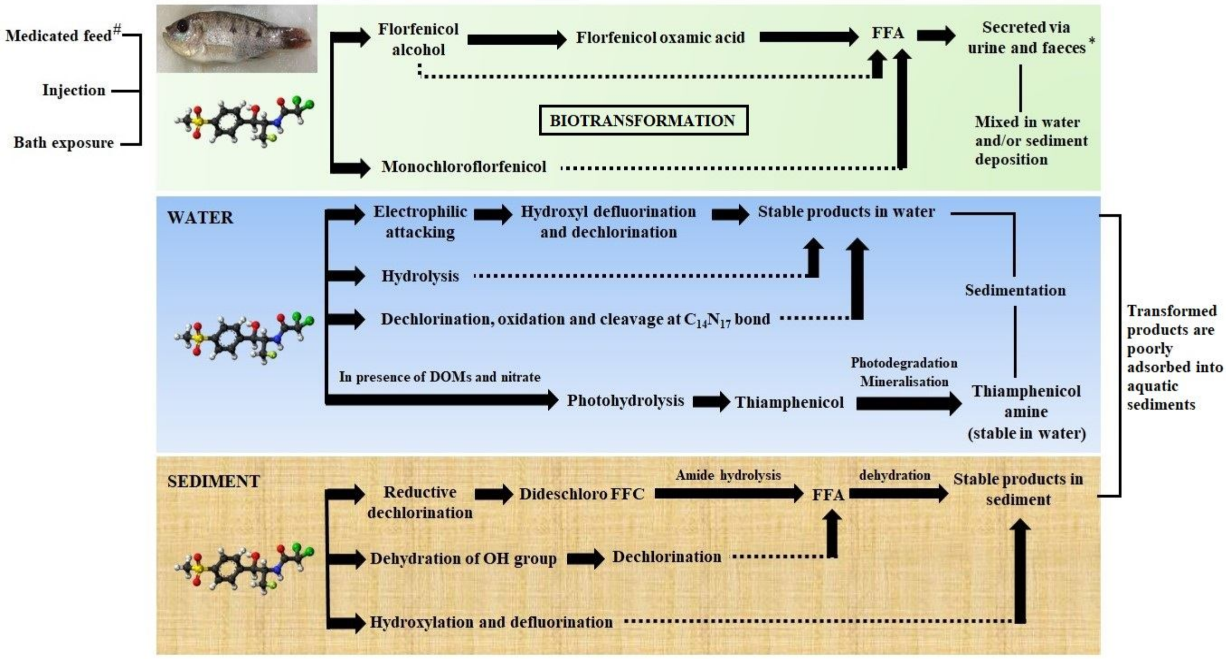
Interestingly, Zhang *et al.* (2023a) revealed that *Pseudomonas* plays a significant role as a bacterium under high FFC concentrations and may transfer FRGs to other aquatic microflora via horizontal gene transmission. Under the influence of FFC stress, *Pseudomonas* emerged as the dominant bacterium and is recognized as a potential host for FRGs. There is growing evidence that antibiotic resistance genes (ARGs) can be enriched locally in antibiotic sediments or water column potentially attributing to the promotion of ARB (Oliver *et al.*, 2023) and probably the primordial impact an antibiotic can have on aquatic microflora. Sun *et al.* (2012) observed a decline in the Shannon diversity index in the aquatic sediment following 30 days of FFC application, indicating a fall in sediment microflora. Further, the diversity indices in the higher treatments than the lower treatments. This efficiently proved the inhibition capacity of FFC in the growth of microbial structural diversity. Given the intricate roles bacteria fulfil within aquatic ecosystems, the prospect of such resistant microflora is a matter of concern. Locations near coasts or industries that are impacted by antibiotic inputs have the potential to serve as sites for the accumulation of bacterial antibiotic resistomes and the exchange of genetic material among groups of microbes (Zhang *et al.*, 2023a). The persistence of drugs in water and sediment can lead to potential biomagnification in the aquatic food chain, causing contamination of non-target organisms and ultimately contributing to the rise of ARBs in the aquatic environment (Zhang *et al.*, 2023a,b). The application of broad-spectrum antibiotics targeting pathogens as well as other aquatic microflora led to dysbiosis and a disruption of normal host-microbiota interactions (Vincent *et al.*, 2019; Sumithra *et al.*, 2022). Apart from inducing dysbiosis in the indigenous microbial community, their prioritized application for addressing bacterial infections has led to the emergence of strains resistant to these agents. This situation is concerning, given the current scenario where numerous strains exhibit multidrug resistance. This concern is heightened within the realm of aquaculture, particularly in certain nations where only a limited selection of antibiotics holds approval for therapeutic usage (Vincent *et al.*, 2019). Apart from FFC, OTC, an approved antibiotic has been known to alter aquatic microflora and their resistance towards the antibiotic (Mog *et al.*, 2020). A transferable plasmid, designated as p3PS10, was isolated from a strain of *Piscirickettsia salmonis* that exhibited resistance to OTC. This plasmid was found to carry functional genes responsible for conferring resistance to a range of antibiotics, including FFC, tetracyclines, aminoglycosides and sulfonamides (Cabello *et al.*, 2023).

Fate of florfenicol in the aquatic environment

According to the criteria presented during the Veterinary International Conference on Harmonization (VICH) and Centre for Veterinary Medicine (CVM) Phase II in which substances with a log octanol-water partition coefficient (log K_{ow}) of <4.0 are not considered bioaccumulative (Shiroma *et al.*, 2021). FFC has a log K_{ow} value of 0.37 (Sun *et al.*, 2023), indicating little potential for bioaccumulation. Given these physicochemical characteristics, it is unlikely that FFC or its metabolites or degradates will accumulate in the environment and biota. Compounds such as FFC have substantial water solubility with an extremely low log K_{ow} and, thus, tend to remain in the water column. FFC has a low molecular weight, as do its metabolites, which range from 69 to 89% of the parent mass (Shiroma *et al.*, 2021). The parent and metabolite solubilities and log K_{ow} values differ. The metabolites are markedly more soluble with solubilities ranging from 49.7 to >500 g/L and are markedly less lipophilic, i.e., have lower log K_{ow} (Sun *et al.*, 2023). Theoretically, these factors make the metabolites even more likely than FFC to enter and remain in water relative to sediment and not to bioaccumulate in biota. In recent years, researchers have focused more on ecological effects and environmental toxicity, while further studies on human health risks are in high demand (Limbu, 2020). FFC is known to degrade easily by the process of dechlorination, amide hydrolysis, photodegradation, hydroxylation, defluorination and many other ways in an aqueous environment (Zhang *et al.*, 2016) and soil (Qiu *et al.*, 2021). Nevertheless, the metabolites are known to be poorly adsorbed in the sediment (Sun *et al.*, 2012). Aqueous FFC degradation focused on abiotic degradation, such as photolysis and hydrogen peroxide (H_2O_2) oxidation (Mitchell *et al.*, 2015; Zhang *et al.*, 2016; Yin *et al.*, 2018). However, the role of dissolved organic matter (DOM) in the aquaculture environment need not be overlooked. The presence of nitrate and DOM could affect the photolysis of FFC in solutions through the light screening effect, quenching effect and photoinduced oxidization process. The degradation pathways of FFC including hydrolysis, oxidation via dechlorination and cleaving of the side chain were all found in the photodegradation of FFC in DOM solutions (Gao *et al.*, 2015). Natural aqueous compositions, e.g., DOM and nitrate, could significantly influence the photochemical fate of organic pollutants in water through energy transfer, charge transfer or oxidation with photo-generated ROS (Gao *et al.*, 2015). Overall, DOM promoted the decay of FFC under irradiation. Consecutively, Qiu *et al.* (2021) demonstrated that the soil is also involved in fluorine and amide hydrolysis, dechlorination, demethylation, fluorine elimination, and nucleophilic hydroxylation. Biodegradation of thiamphenicol and chloramphenicol showed that hydrolysis was the characteristic pathway for amphenicol antibiotics, while dechlorination and then decarboxylation was also possible (Ma *et al.*, 2019; Song *et al.*, 2020). Dehydration and reduction of the sulfonyl group were reported for the biodegradation of amphenicol antibiotics (Mitchell *et al.*, 2015).

Transformation products (TPs) of florfenicol

In veterinary medicine, there are four TPs of FFC, among which only FFA is reported in aquaculture (Bardhi *et al.*, 2023). FFC biotransformation is known to happen via two pathways. The first pathway follows dechlorination, leading to the formation of monochloro florfenicol, which eventually transforms into FFA. The second pathway follows hydroxylation, leading to the formation of florfenicol alcohol, followed by acetylation (florfenicol oxamic acid) and eventually FFA. However, FFC biodegradation in the soil and aqueous environment is known to create a lot of TPs, among which FFA (TP247) and dideschloro FF (TP289) were observed to proliferate (Zhang *et al.*, 2016; Qiu *et al.*, 2021). Nevertheless, FFA was known to undergo rapid dehydration, leading to the formation of TP229, which was also detected in plenty (Qiu *et al.*, 2021). The frequencies of detection and intensities of TP247 and TP229 suggested that these two were the most important TPs of FFC in the environment, which was consistent with the results obtained in the lab incubation (Qiu *et al.*, 2021). A tentative pathway of FFC post-dietary-administration in fish is illustrated in Fig. 1. FFC has stable physical and chemical properties, which are difficult to break down, and a considerable amount of unabsorbed FFC is



[*Apart from florfenicol amine (FFA), FFC can also be secreted out in its parent form and non-transformed. #Leaching of FFC from the medicated feed when administered into the water has also to be accounted for.]

Fig. 1. Tentative pathway of degradation of florfenicol (FFC) in the aquatic environment post-administration in fish via medicated feed, injection or bath exposure

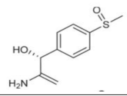
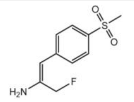
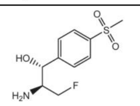
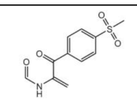
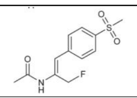
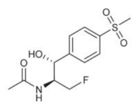
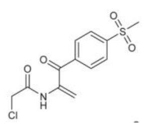
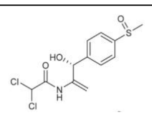
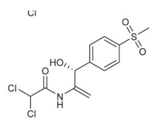
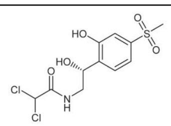
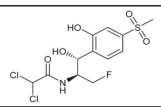
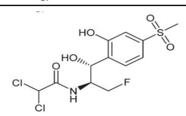
regularly released, culminating in its persistence in the environment. Thus, the leftover antibiotics in the environment undoubtedly influence microorganisms, resulting in the emergence of FFC resistance. According to published studies, FFC therapy altered the composition of the microbiota and lowered biodiversity by functioning as a significant stressor (Bojarski *et al.*, 2020; Sumithra *et al.*, 2022). FFC treatment encouraged the spread of FRGs and resulted in mutation-driven resistance (Zhang *et al.*, 2022). The effect on bacterial community composition in water was more significant than that in sediment (Jara *et al.*, 2021; Zhang *et al.*, 2022). High FFC concentrations disrupt the original community structure, significantly increasing the relative prevalence of ARBs (Zhang *et al.*, 2022).

The TPs are also a matter of concern in the aquatic environment. They can be toxic or may demonstrate antibacterial properties both in the water and soil (Zhang *et al.*, 2016; Qiu *et al.*, 2021). However, the effect of TPs on piscine physiology has been sparsely studied and warrants extensive research. Aqueous photodegradation intermediates of organic pollutants could increase the toxicity toward living organisms, while complete mineralization may decrease the effects of toxicity (Jiao *et al.*, 2008). It may be possible that an increase in toxicity during the photodegradation of FFC can occur in the aquatic environment. This hypothesis was based on the fact that photodegradation products stemming from chain cleavage may result in lower steric resistance and easier penetration into the cells of organisms. Hence, studies focusing on the toxicity of photoproducts of FFC are needed. Qiu *et al.* (2021) estimated the environmental persistence, antibacterial activities and aquatic toxicities of the 12 identified TPs by BIOWIN3 (EPI Suite V4.11), AntiBac-Pred and ECOSAR V2.0, respectively. They proposed that all TPs might have similar or shorter environmental persistence than FFC, and all TPs might persist in the environment from weeks to months. TPs with larger molecular masses, i.e., TP337, TP339, TP341, and TP373, might have antibacterial activities, especially towards Gram-negative human enteric bacteria. Moreover, the antimicrobial spectra of these TPs were very similar to that of FFC. The structural similarity between the TPs and FFC might be the cause of the TPs' antibacterial activities. The aquatic toxicities of TPs were estimated to be low, at mg/L level (Sun *et al.*, 2012), which should be much higher than their environmental concentrations. Therefore, extensive studies are obligatory.

FFC is shown to be relatively stable in water under ambient conditions, and it takes a considerable amount of time to hydrolyze in water (pH 7) at 60°C (Mitchell *et al.*, 2015). The estimated half-life of FFC was enumerated to be 38.1 days at the aforesaid conditions.

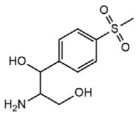
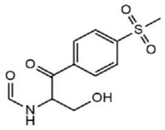
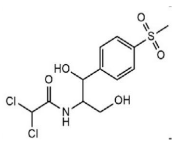
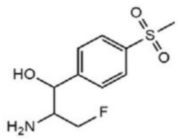
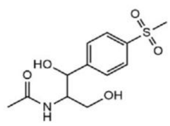
FFC is also known to be significantly more reactive under alkaline conditions (pH 9), depicting a half-life of only 2.3 days (Mitchell *et al.*, 2015). The investigation into the degradation of FFC in aquatic waters revealed intriguing insights into the fate of this antibiotic. Through the use of Total Ion Chromatograms (TICs), researchers discerned the emergence of various degradation products, shedding light on the complex pathways of FFC transformation. In particular, the analysis unveiled the presence of five distinct degradation products for FFC (Mitchell *et al.*, 2015). The documented TPs of FFC in the aquatic environment upon photo-, hydro- or pedological degradation are summarized in Table 2. The identified masses of these

Table 2. Transformed products of florfenicol upon degradation or biotransformation in the aquatic environment

Name	Monoisotopic mass	Structure	Process	References
TP211	211.27		Dehydrogenation of dichloroacetic acid group	Qiu *et al.*, 2021
TP229	229.05		Deacetylation	
TP247 (Florfenicol amine)	247.06		Amide hydrolysis	
TP253	253.04		Dechlorination, dehydration and decarboxylation	
TP271	271.06		Dehydration and dechlorination	
TP289 (Dideschloro FFC)	289.07		Reductive dechlorination	
TP301	301.01		Dehydrogenation and dechlorination	
TP321	320.99		Reduction of the sulfonyl group	
TP337	336.99		Dehydrogenation and defluorination	
TP339	338.98		Dehydration of hydroxyl group	
TP341	340.98		Defluoromethylation	
TP373	372.99		Electrophilic substitution by a hydroxyl group on the benzene ring	

Cont. Table 2.

Table 2., Cont. ...

Name	Monoisotopic mass	Structure	Process	References
NA	246.10		Abiotic hydrolysis	Mitchell *et al.*, 2015
NA	272.10		Alkyl fluoride hydrolysis	
NA	356.00			
NA	248.10			
NA	288.10			

TP: Transformed products; FFC: Florfenicol; NA: Not available

degradation products closely aligned with those anticipated from hydrolysis of the amide moiety, suggesting a significant role of this process in FFC degradation (Table 2). Furthermore, an additional avenue of degradation for FFC was indicated by the masses detected, which pointed towards alkyl fluoride hydrolysis. Notably, one of the primary hydrolysis products stemming from FFC degradation (TP356) exhibited an identical mass to that of thiamphenicol (Table 2). Thiamphenicol shares a similar chemical structure with FFC, with the exception that a fluoride atom is substituted with a hydroxyl group (Mitchell *et al.*, 2015). Intriguingly, thiamphenicol possesses a higher minimum inhibitory concentration (MIC) for aquatic species in comparison to FFC (Ho *et al.*, 2000). This observation implied that the hydrolysis products resulting from alkyl fluoride degradation of FFC might retain bioactivity, while potentially exhibiting reduced toxicity within aquatic environments. The ramifications of amide hydrolysis on the toxicity of these degradation products remain to be entirely determined. Nonetheless, the precedent set by secondary amide hydrolysis deactivating ß-lactam antibiotics offers a compelling analogy (Testa and Mayer, 2003). This suggested that a similar deactivation process could potentially occur with FFC. In essence, the degradation of FFC in water is a multi-faceted phenomenon involving intricate pathways such as amide hydrolysis and alkyl fluoride degradation. The photolysis of FFC under the influence of solar radiation has also been studied. The four pathways, viz., electrophilic attacking at the –OH site of the benzene ring, hydrolysis at the –F site, dechlorination and then oxidation and cleavage of the C_{14}-N_{17} bond have been reported (Zhang *et al.*, 2016). Under irradiation, photohydrolysis generated a TP similar to TP373 with a monoisotopic mass of 372, the name of which was proposed as MW372 by Zhang *et al.* (2016). Alike, Zhang *et al.* (2016) observed the cleavage of the C_{19}-C_{20} bond, followed by –OH subtraction and H addition leading to two TPs, viz., MW273 and MW257, which were closely similar to those reported by Qiu *et al.* (2021). Further investigation into the bioactivity and toxicity of these products is warranted to comprehensively understand the fate of FFC in aquatic environments.

Understanding the fate of FFC in aquatic ecosystems is of paramount significance in light of its potential ecological and anthropogenic implications. As a prominent antibiotic deployed in aquaculture, FFC incursion can engender the emergence of ARB, thereby perturbing microbial consortia and altering ecological dynamics. Such perturbations could potentially disturb nutrient cycling processes, resulting in cascading effects on aquatic trophic structures and overall ecosystem function. The genetic determinants encoding AMR in FFC-exposed environments raised concerns about the horizontal transfer of ARGs among bacterial populations. This genetic flux not only amplified the reservoir of ARGs in aquatic settings but also bore implications for human health, as ARGs can traverse environmental reservoirs and potentially impact clinical settings. The intricate interplay of FFC in aquatic systems necessitated a comprehensive investigation of its fate, encompassing its degradation kinetics, metabolite formation, and potential bioaccumulation pathways. A rigorous understanding of these aspects is pivotal not only for designing effective regulatory frameworks but also for advancing our broader comprehension of pharmaceutical persistence and ecological resilience within aquatic environments.

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Author's contribution: AB: Data and literature collection, data formulation, writing; TJA: Data and literature collection, reviewing and editing.

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