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Evaluation of Oxalinic Acid in Aquaculture: Therapeutic Utility, Histopathological Consequences, and Residue Risks

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Abstract
Oxolinic acid (OA) is one of the broad-spectrum quinolones that is a critically important medicine for humans and is used as a second-line treatment in aquaculture at 12 mg/kg biomass/day for 7 consecutive days. In both freshwater and saltwater aquaculture, oxolinic acid is given through medicated feed and bathing treatments, with positive clinical results seen at low dosage rates. Against the diseases that are most commonly found in fish farms, oxolinic acid exhibits strong antibacterial action with effective efficacy. Minimum inhibitory concentrations (MIC) values of oxolinic acid also were markedly lower than the corresponding MIC values of other quinolones antibiotics. The present review study was also evaluate the therapeutic effects of oxolinic acid on biochemical and histopathological alterations as well as the residues levels in different organs of fish. A significant alteration in plasma glucose, creatinine, plasma calcium and chloride levels, alkaline phosphatase, alanine transaminase and aspartate transaminase was observed during the dosing period of medicines. On the other hand, this review study also interpreted the mild to marked histopathological changes in gills, kidneys, liver and spleen during the dosing phase of drugs, indicating the possible toxic effects of OA on different organs of fish. This review study also interpreted that the therapeutic dose group's tissue OA residue levels were well under the European Medicines Evaluation Agency's (EMEA) maximum residue limit (MRL).

Keywords: Oxolinic acid, Minimum inhibitory concentrations, Maximum residue limit, Histopathology

Introduction
Oxolinic acid is a first-generation quinolone antibiotic that is used only under exceptional circumstances. Oxolinic acid has been used extensively to treat several systemic bacterial infections in fish (Austin *et al*., 1983; Rodgers and Austin, 1983). Oxolinic acid was first described in 1968 and is effective against Gram-negative bacteria including many fish pathogens. The history of the use of quinolone, and oxolinic acid, in Europe is well documented. It was identified as being very useful in the control of furunculosis in salmonids in Europe in 1983 (Austin *et al*., 1983), although it had been used earlier in Japan (Inglis, 2000). The compound was used successfully for the control of furunculosis (Endo *et al*., 1973; Austin *et al*., 1983), vibriosis (Endo *et al*., 1973; Austin *et al*., 1982) and enteric red mouth disease (Rodgers and Austin, 1983). Oxolinic acid is the most used antibacterial agent in Norwegian aquaculture (Grave *et al*., 1999). First-generation quinolones such as oxolinic acid and nalidixic acid are bactericidal and act by selectively inhibiting prokaryotic topoisomerase II (DNA gyrase), an enzyme that induces topological changes necessary for replication, recombination, and transcription of bacterial (circular) chromosomal DNA

(Drlica and Coughlin, 1989; Zimmer *et al*., 1990). Oxolinic acid belongs to the group of 4-quinolones and its mode of action is to interfere with the bacterial DNA gyrase, thus inhibiting the supercoiling of DNA (Lundén and Bylund, 2000). This antibiotic has low binding properties to plasma proteins and possesses good solubility in biological membranes, resulting in good tissue penetration (Bjorklund and Bylund, 1991). Oxolinic acid is active against both Gram-negative and Gram-positive bacteria although it is used almost exclusively for its Gram-negative microbiological activity (Skjolstrup *et al.*, 2000). Although more recent additions to the quinolone drug family out-perform oxolinic acid concerning both bactericidal activity and bioavailability, its relatively modest cost, low mammalian and fish toxicity and satisfactory performance render it a useful and widely used drug, particularly in the aquaculture industry (Skjolstrup *et al.*, 2000).

Dosage and efficacy

Quinolones, such as oxolinic acid, which constitute an antimicrobial family characterized by a broad spectrum, high potency and theoretically low occurrence of side effects, are commonly used in aquaculture (Lin *et al*., 2010; Sturini *et al*., 2014; Zeghioud *et al*., 2019). Oxolinic acid is administered through both medicated feed and bathing treatments and good clinical effects at low dosage rates are observed in both fresh and saltwater aquaculture (Lai and Lin, 2009). Oxolinic acid is administered to fish mixed in the feed usually for 7 days at 12 mg/kg body weight/day (EMEA, 2005). According to Samuelsen and Bergh (2004), the medicated feeds were made by mixing oxolinic acid (100% pure) with commercial feeds at a drug dosage of 20 mg/kg fish. The drugs were coated onto the surface of the pellets using capelin oil. According to Liao *et al*. (1992) to control such diseases, oxolinic acid is applied at a dose of 20-40 mg/kg body weight mixed with feed and administered to fish daily for 5 days. In Philippine grow-out ponds, OTC, oxolinic acid, chloramphenicol and furazolidone have been incorporated into artificial feeds as treatments against luminous vibriosis (Cruz-Lacierda *et al*., 2000). Oxolinic acid, already registered as a fish therapeutant in Japan and several European countries for *A. salmonicida* and *Yersinia ruckeri* infections is efficacious in-vitro against select isolates of *Vibrio* spp. (*V. anguillarum, V. tubiashn. V. cercariae, V. damsela), Aeromonas* spp. *(A. hydrophilia, A. sobria, A. caviae, A. salmonicida), Pasteurella piscicida, P. fluorescens. Y. ruckeri* and *E. tarda* with MICs ranging from <0.075 to 0.3 µg/mL, except for *V. tubiashn* (1 µg/mL), and *P. fluorescens* (3 µg/mL) (Ledo *et al*. 1987). The efficacy of oxolinic acid was investigated with 10 isolates of *A. salmonicida* and *A. hydrophila* in which doses of 1, 10, and 100 µg/mL were bactericidal (Austin *et al*. 1983), and with 25 isolates of *Y. ruckeri* in which it was bacteriostatic at 1 µg/mL and bactericidal in 23 of 25 (92%) isolates at 5 µg/mL (Rodgers and Austin, 1983).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antibiotic/antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Oxolinic acid has highly potent antibacterial activity against the pathogens most frequently encountered in fish farms. Oxolinic acid has a high activity against *A. salmonicida*. The MIC values ranged between 0.0075 and 0.03 µg/mL for 10 isolates tested. These MIC values were markedly lower than the corresponding MIC values for nalidixic acid, which ranged between 0.06 and 0.125 µg/mL (Neussel and Linzenmeier, 1973). Endo *et al.* (1973) also showed that oxolinic acid had a MIC of 0.02 µg/mL against the one strain of *A. salmonicida* tested and also demonstrated the MIC of oxolinic acid against the 7 strains of *A. liquefaciens,* tested ranged between 0.02 and 0.09 µg/mL and against the 5 strains of *V. anguillarum* tested with a range of 0.02 - 0.09 µg/mL. Samuelsen and Bergh (2004) observed the MIC value for *V. anguillarum* strain HI-610, of 0.016 mg/mL against oxolinic acid. The

MIC against strains of *Vibrio* ranged from 0.015 to 0.5 µg/mL for oxolinic acid (Samuelsen and Lunestad, 1996).

Impact of oxolinic acid on organ histology

Histological effects provide a rapid diagnosis to detect anomalies in several fish tissues and organs after antibiotic exposure. The liver is the main organ for bioaccumulation, metabolism and detoxification. Hepatotoxicity in carp was observed due to the toxic effect of quinolones leading to an increase in the area of liver cells and nuclei and found that carp liver fat tissue in the antibiotic's treatment group was significantly more than that in the control group, which confirmed the mutagenesis of quinolones on carp liver (Zhang *et al.,* 2021). Due to the toxicity of oxolinic acid in *O. mykiss*, ulceration in the liver was observed. Changes such as an almost 2.5-fold increase in the volume of the rough endoplasmic reticulum led to dilation, vesiculation and fragmentation of the liver. Glycogen deposits were depleted to approximately 50% of their control volume accompanied by the formation of clusters of lipid droplets and mitochondrial hyperplasia due to the toxicity of oxolinic acid (Moutou *et al.*, 1997). The toxicity of quinolones with alterations in gill morphology, liver and kidney was observed in *Cirrhinus mrigala* (Ramesh *et al.*, 2021). Changes in gill-like lamellar fusion, epithelial lifting, loosening of the primary gill bar and cytoplasmic vacuolation were documented by them. Such alterations resulted in the impairment of respiratory function that could result in the failure of an adequate supply of oxygen and inorganic ions for normal metabolic activities of fish (Ramesh *et al.*, 2021). In the kidney, tubular necrosis, fragmented glomerulus, vacuolation, degeneration and hypertrophy were noted due to the excretion of metabolized quinolones (Ramesh *et al.*, 2021). Also, several anomalies in the kidney such as thickening of Bowman's capsule, tubular cell necrosis, shrinkage of glomeruli, tubular degeneration, glomerular necrosis, hyaline droplets degeneration, cloudy swelling, congestion in the renal parenchyma, reduction of lumens and renal tubular separation were noticed (Ramesh *et al.*, 2018). Hsu *et al.* (1993) documented histological alterations in gastrointestinal and spleens by the toxicity of quinolones in *O. mykiss* fingerling. Histological alterations such as an increased number of eosinophilic granular leukocytes in the gastrointestinal sub mucosa were observed in fish. In spleen of antibiotics-treated fish appeared to be more congested than those in the control animals. Also, mild peracute to acute foci of myocyte necrosis were observed with no associated inflammatory response and small clusters of melanocytes (Hsu *et al.*, 1993).

Impact of oxolinic acid on plasma biomarkers

Antibiotics are known to cause stress on the fish body (Refaey and Li, 2018). Stress response in fish leads to diverse enzymatic pathways and cellular turnovers (Refaey and Li, 2018). Additionally, fish respond to stress by lifting the contents of circulating catecholamines and corticosteroids (Yousefi *et al.*, 2016). The elevation of stress hormones leads to increased glucose and proteins, which are detectable in serum and plasma (Yousefi *et al.*, 2016). Fish liver and kidney are considered to be the organs of metabolic homeostasis (Hernández-Pérez *et al.*, 2019). Stress, both chronic and acute, affects these organs directly. Conversely, enzymatic responses from the liver accumulating in serum (aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT)) have been considered a major biomarker of stress (Hernández-Pérez *et al.*, 2019). Ramesh *et al.* (2018) observed increased AST and ALT activities in the plasma of *C. carpio* during the time of quinolone antibiotic treatments. The AST and ALT are the liver guiding enzymes and function as catalysing in transferring amino groups to alpha-keto acids and aspartic acid to α-ketoglutaric acid in the interconversion of carbohydrates and proteins Ramesh *et al.* (2018). Van der Oost *et al.* (2003) stated that changes in the activity of plasma AST and ALT act as a sensitive indicator to know the health status of the organs of fish exposed to chemicals. The enzyme activity may increase in the blood (extracellular fluid) when there is minor cell damage. An increase in plasma AST and ALT activity is an indication of functional damage of muscular, hepatic, and renal damage. However, AST and ALT levels in an organism depend on protein and carbohydrate metabolism. Any changes in the protein and carbohydrate metabolism may also lead to a change in the transaminase activity (Van der Oost *et al.*, 2003).

Plasma glucose is the most important stress indicator parameter in fish. Changes in blood glucose concentrations are most often associated with renal injury (Wang *et al.*, 2021). Plasma concentrations of glucose are regulated by complex interactions of hormones such as glucagon and cortisol. An increase in plasma glucose concentrations can cause a hyperglycemic condition (Agrahari *et al.*, 2007). Plasma ALP splits various phosphorous esters at alkaline pH and its activity is related to cellular damage. Increased activity of plasma ALP in blood plasma is an indicator of hepatic tissue damage with disturbed normal liver function and dysfunction (Agrahari *et al.*, 2007). Zhang *et al.* (2021) observed that significant changes occur in biochemical indicators of the quinolones treatment group of *C. carpio* compared to those of the control including the level of plasma glucose, AST and ALT. Plasma glucose levels increased during the time of treatment of quinolones antibiotic due to the increases in stress level. Along with plasma glucose levels, ALT and AST levels also were increased in quinolone treatment groups compared to the control. Rizkalla *et al.* (2003) observed that plasma glucose levels were significantly elevated during the administration of quinolone antibiotics in catfish *Clarias gariepinus.* They also observed that the plasma AST and ALT activities also increased due to the administration of quinolones in *C. gariepinus.* Plasma ALP levels also increased in the antibiotics-treated groups compared to the control group.

Inorganic ions are minerals found as ions in body fluids, including blood. Inorganic ions, such as calcium (Ca+) and chloride (Cl^-) routinely used to determine the health status of an organism (Ramesh *et al.*, 2021). The Cl^- is very much essential for the maintenance of energy expenditure during normal or stressful conditions in an organism. Particularly, freshwater animals compensate for their renal and surface loss of ions (mainly Cl^-) by absorbing the ions from the external medium and maintaining their normal physiological process and body fluid homeostasis with the help of ion/ osmoregulatory processes. Inorganic ions play a vital role in osmoregulation and homeostasis (Ramesh *et al.*, 2021). Inorganic ion homeostasis is necessary for any organism to remain healthy and any alteration in this level in fish plasma leads to cardiovascular collapse and finally death. Ramesh *et al.* (2021) observed a decrease in the levels of plasma inorganic ions such as calcium (Ca^+) and chloride (Cl^-) in the quinolones treatment group, which revealed that quinolones could interfere with the membrane-bound enzyme activity in the gill tissues.

Oxolinic acid residues in fish

When high concentrations of antibiotics were used, their residues have been found in the blood and the other tissues of the animals. However, since the antibiotics can be rapidly eliminated, disappear from both blood and tissue within a few days after the animals are placed on non-medicated feed (Matsunaga and Hayakawa, 2018). Direct contamination of animal-derived feed may occur from air and water during processing, storage and transportation. Antibiotics in feed given to animals are examples of indirect contamination (Matsunaga and Hayakawa, 2018). Kasuga *et al.* (1984) and Ueno *et al.* (1988) in their works on *O. mykiss* observed complete depletion of oxolinic acid residues within 21 days after cessation of oxolinic acid dosing in the liver, muscle and other tissues. Ueno *et al.* (1988) also observed that in *S. salar* the residues of oxolinic acid were completely depleted within 30 days after cessation of dosing in the liver, kidney blood and muscle tissues. In *Sparus* *aurata*, Rigos *et al.* (2003) observed that the highest concentration of oxolinic acid was found in the liver (2660 ng/g), followed by bile (2370 ng/ml), skin (2350 ng/g), muscle (1330 ng/g) and plasma (880 ng/ml). Rigos *et al.* (2003) also found a similar result in *Diplodus puntazzo* organ such as the highest concentration in the liver (2720 ng/g), followed by bile (2360 ng/ml), skin (1960 ng/g), muscle (1770 ng/g) and plasma (910 ng/ml). Tendencia (2022) found that oxolinic acid accumulation was highest in the muscle (0.11+0.06 µg/g) compared to plasma (0.005+0.0001 ug/mL) in pompano fish, *Trachinotus blochii*. Song *et al.* (2022) demonstrated with quinolones on their accrual and depletion in carp *C. carpio haematopterus* treated with a dose of 10 mg /kg biomass/day for 5 days, residues reached a peak level of 535.00±0.236 µg/kg on day 1 of administration.

Conclusion

In conclusion, the extensive usage of oxolinic acid, a first-generation quinolone antibiotic, in aquaculture underscores its importance in combating systemic bacterial infections in fish. Despite being a first-generation quinolone, oxolinic acid remains a significant therapeutic option due to its effectiveness against a broad spectrum of Gram-negative bacteria, particularly in controlling diseases like furunculosis, vibriosis, and enteric red mouth disease. Its relatively low cost, coupled with favorable toxicity profiles for both mammals and fish, contributes to its widespread use, especially in the aquaculture industry.

Dosage studies reveal the efficacy of oxolinic acid in medicated feed formulations, showing good clinical outcomes at low dosage rates in both freshwater and saltwater aquaculture settings. Additionally, investigations into its minimum inhibitory concentration (MIC) against prevalent fish pathogens demonstrate its potent antibacterial activity, notably against *Aeromonas salmonicida* and *Vibrio* species.

However, the impact of oxolinic acid on fish organ histology and plasma biomarkers warrants attention. Histological examinations reveal hepatotoxicity, gill and kidney anomalies, and gastrointestinal and spleen alterations, emphasizing the potential adverse effects on fish health. Moreover, alterations in plasma biomarkers such as glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels indicate stress and organ damage in fish exposed to oxolinic acid.

Furthermore, the persistence of oxolinic acid residues in fish tissues raises concerns about food safety and environmental impact. Although residues are depleted within a few weeks after cessation of medication, their initial accumulation underscores the need for prudent antibiotic usage in aquaculture practices.

In summary, while oxolinic acid remains a valuable tool in managing bacterial infections in aquaculture, its usage should be carefully monitored to mitigate potential adverse effects on fish health and ensure food safety standards are met. Continued research into alternative treatments and antibiotic stewardship strategies is crucial to sustainably address disease challenges in aquaculture while minimizing environmental and public health risks.

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