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Progress of research on the toxicology of antibiotic pollution in aquatic organisms

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ABSTRACT

Antibiotics are widely used to improve human and animal health and treat infections. Antibiotics are often used in livestock farms and fisheries to prevent diseases and promote growth. Recently, there has been increasing interest in the presence of antibiotics in aquatic environments. Low levels of antibiotic components are frequently detected in surface water, seawater, groundwater, and even drinking water. Antibiotics are consistently and continually discharged into the natural environment as parent molecules or metabolites, which are usually soluble and bioactive, and this results in a pseudo and persistent pollution. The effects of environmental antibiotic toxicity on non-target organisms, especially aquatic organisms, have become an increasing concern. Although antibiotics have been detected worldwide, their ecological and developmental effects have been poorly investigated, particularly in non-target organisms. This review describes the toxicity and underlying mechanism of antibiotic contamination in aquatic organisms, including the effects on vertebrate development. A considerable number of antibiotic effects on aquatic organisms have been investigated using acute toxicity assays, but only very little is known about the long-term effects. Aquatic photosynthetic autotrophs, such as *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae*, and *Lemna minor*, were previously used for antibiotic toxicity tests because of low cost, simple operation, and high sensitivity. Certain antibiotics show a different degree of potency in algal toxicity tests on the basis of different test algae. Antibiotics inhibit protein synthesis, chloroplast development, and photosynthesis, ultimately leading to growth inhibition; some organisms exhibit growth stimulation at certain antibiotic concentrations. *Daphnia magna* and other aquatic invertebrates have also been used for checking the toxicity priority of antibiotics. When investigating the acute effect of antibiotics (e.g., growth inhibition), concentrations in standard laboratory organisms are usually about two or three orders of magnitude higher than the maximal concentrations in the aquatic environment, resulting in the underestimation of antibiotic hazards. Vertebrate organisms show a promising potential for chronic toxicity and potentially subtle effects of antibiotics, particularly on biochemical processes and molecular targets. The adverse developmental effects of macrolides, tetracyclines, sulfonamides, quinolones, and other antibiotic groups have been evaluated in aquatic vertebrates such as *Danio rerio* and *Xenopus tropicalis*. In acute toxicity tests, low levels of antibiotics have systematic teratogenic effects on fish. The effects of antibiotics on oxidative stress enzymes and cytochrome P450 have been investigated. Cytotoxicity, neurotoxicity, and genotoxicity have been observed for certain antibiotic amounts. However, there are no firm conclusions regarding the chronic toxicity of antibiotics at environmentally relevant levels because of the lack of long-term exposure studies. Herein, future perspectives and challenges of antibiotic toxicology were discussed. Researchers should pay more attention to the following points: chronic toxicity and potentially subtle effects of environmentally relevant antibiotics on vertebrates; effects of toxicity on biochemical processes and mode of action; combined toxicity of antibiotics and other antibiotics, metabolites, and heavy metals; and environmental factors such as temperature and pH.

1. Introduction

Various antibiotics are widely and clinically used as over-the-counter (OTC) drugs with usually broad antimicrobial spectrum. Large amounts of antibiotics are also used to promote animal growth, inhibit bacteria reproduction, prevent and cure diseases in aquaculture and livestock farm. The huge consumption of common antibiotics and the trait that most of the active antibiotics and their metabolites are water soluble lead to the pseudo and persistent pollution in aquatic environment and potential threat to ecosystem including human. The traditional view that antibiotics inhibit the bacteria growth and proliferation with no harm to the host is twisted now. Increasing evidence suggests that the parent antibiotics and metabolites discarded into surroundings

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are bioactive and persistent in low concentration (ng/L–μg/L) (Table 1), posting a potential hazard in food-chain system [12].

In 2013, the usage of 36 common antibiotics in China was 92,700 tons, among which 54,000 tons was excreted by human and animals, and an overwhelming majority entered into the surroundings [13]. Antibiotics are frequently detected in natural environment from different nations and regions, grabbing the attention to the toxicity in non-target organisms. Blue algae are sensitive to most antibiotics while daphnids and fish are more sensitive to macrolides [14]. An ecological risk assessment of over 226 antibiotics [15] suggests that 20% antibiotics are very toxic to algae (EC_{50} < 1 mg/L), while 16% were extremely toxic (EC_{50} < 0.1 mg/L) and 44% very toxic to daphnids. Meanwhile >50% antibiotics are predicted to be toxic (EC_{50} < 10 mg/L) and one third very toxic to fish. The research grabs attention to ecological risk of antibiotic pollutions on non-target organisms and suggests that sensitivity of different species to antibiotics varies. Certain antibiotics might show different toxicity between autotroph and heterotroph, or between lowly forms and higher lives.

Therefore, based on test species and antibiotic structures, we reviewed the toxicology of common antibiotics on autotrophic hydrophytes (algae mainly), aquatic invertebrates and aquatic vertebrates. The three groups of organisms represent different nutrition and metabolism pathways, showing distinct different response to antibiotic pollution.

2. Toxicity of antibiotics on algae

With low cost, high sensitivity and simplicity in manipulation, algae are one of the first applied to ecological risk assessment of antibiotics.

2.1. Ecotoxicity assessment and sensitivity rank analysis of antibiotics on algae

Initially classical toxicity assessment tests of antibiotics based on algae are conducted with median effective concentration (EC_{50}) or median lethal concentration (LC_{50}) as main content. For example, Yang et al. [16] evaluated and ranked the growth inhibiting effects of 10 antibiotics such as roxithromycin and clarithromycin on *Selenastrum capricornutum Pseudokirchneriella subcapitata* based on the findings of 72 h-EC_{50}, whose values varied from μg/L to mg/L. With longer exposure time of 7 days, the EC_{50} values of *Lemna minor* to erythrocin and tetracycline are 5.6 g/L and 4 g/L [17], separately, higher than that of microalgae (μg/L–mg/L). The variety of toxicity parameters in different test species suggests the species diversity in toxic sensitivity to certain antibiotics. For example, the cyanobacterium *Anabaena flosaquae* and macrophyte *Lemna minor* exhibit higher sensibility to fluoroquinolones antibiotics than the freshwater microalga *Desmodesmus subspicatus* or the dicotyledonous macrophyte *Myriophyllum spicatum* [18].

Table 1
Prominent aquatic antibiotics and their environmental concentrations.

Major groups	Typical antibiotic	Environmental concentration
β-Lactams	Lincomycin	Surface water 248.9 ng/L [1]
Aminoglycosides	Gentamicin	Sewage plant export 1300 ng/L [2]
Macrolides	Erythromycin-H_2O	Surface water 1700 ng/L [3]
	Clarithromycin	Surface water 260 ng/L [4]
	Roxithromycin	Surface water 560 ng/L [4]
Tetracyclines	Tetracycline	Underground water 3.8 ng/L [5]
	Oxytetracycline	Surface water 340 ng/L[1] [3]
	Chlortetracycline	Surface water 690 ng/L [3]
Sulfonamides	Sulfamethoxazole	Surface water 1900 ng/L [3]
		Underground water 470 ng/L [4]
	Sulfamethizole	Surface water 130 ng/L [3]
	Sulfamethazine	Surface water 4660 ng/L [6]
		Underground water 160 ng/L [4]
	Sulfadoxine	Surface water 460 ng/L [6]
Quinolones	Ciprofloxacin	Sewage plant export 260 ng/L [7]
		Surface water 185 ng/L [8]
	Norfloxacin	Surface water 208 ng/L [9]
	Ofloxacin	Surface water 89 ng/L [9]
		Sewage plant export 210 ng/L [10]
	Enrofloxacin	Livestock farm export 680 ng/L [11]
		Underground water 3 ng/L [5]

2.2. The toxicity of antibiotics on algae

The toxic effects of antibiotics including tetracyclines [19], sulfonamides [20] and macrolides [17] and many other antibiotics on algae present mainly as adverse impact of growth and development, especially growth inhibition. For example, long-term exposure of sulfathiazole might induce growth inhibition on the macroalgae *Ulva lactuca* [21] and *Lemna gibba* [22], while tetracyclines, oxytetracycline and aureomycin would influence the cell growth in *Microcystis aeruginosa* [23]. An underlying mechanism for antibiotics to inhibit growth is the inducible production of abscisic acid [17]. Sulfonamides are reported to inhibit growth via influencing chlorophyll biosynthesis pathway [20].

Growth inhibiting is not the only effect of antibiotics on algae. Certain concentration of cefradine can stimulate the growth of *Selenastrum capricornutum*. Another example is the hormesis of cefalexin to the same algae, namely, promoting growth in low dosage and inhibition in high dosage [19]. Similarly, 0.001–0.1 μg/L erythrocin could stimulate the growth and photosynthesis while higher levels have the opposite effects to *Microcystis flos-aquae* [24].

Antibiotics affect algae photosynthesis via inhibiting chloroplast formation and protein biosynthesis [25] and damaging chlorophyll [26]. The decrease of chlorophyll weakens the capacity of photosynthesis and metabolism, resulting in inhibition of cell proliferation and growth. Hydrophyte containing chlorophyll is susceptive to even low levels of sulfonamide antibiotics. The EC_{50} of sulfamethoxazole to *Synechococcus leopoliensis* is reported as 0.0268 mg/L [20], far below the sulfamethoxazole concentrations in surroundings.

Oxidative stress defense response is induced in algae when exposed to antibiotic pollution. Norfloxacin affects the antioxidative enzymes activity such as catalase (CAT) and glutathione S-transferase (GST) in a dosage-dependent manner [27]. Oxytetracycline (μg/L) is also reported to induce CAT and peroxidase activity changes significantly to activate enzymatic defense in aquatic plant [28].

Recently, Liu et al. [29] identified the candidate target proteins and inferred that the cellular biosynthesis process and the metabolism pathway were involved in the proteomic responses of cyanobacteria (*Microcystis aeruginosa*) exposed to amoxicillin. While this is the first study on the proteomic response of cyanobacteria to antibiotics, no more verification and further research on the key target proteins were conducted in this study.

2.3. Joint toxicity of antibiotics on algae

Mixture of antibiotics might pose joint toxicity. Simple additive effects can be found in binary mixtures of a variety of antibiotics such as sulfonamides and other antibiotics or tylosin and macrolide antibiotics. Synergistic effects come from the binary mixtures of the same class or some combined drugs such as trimethoprim and sulfonamides [16]. Moreover, antagonistic effects are resulted from binary mixtures of tetracycline and 7-aminocephalosporanic acid, a main degradation product of beta-lactam antibiotic cephalosporin [19].

3. Toxicity of antibiotics on aquatic invertebrates

Aquatic invertebrate *Daphnia magna* is a popular species for the contaminant toxicity test. Related standard test guidelines such as *Daphnia magna* Acute Immobilisation Test (OECD 202) and *Daphnia magna* Reproduction Test (OECD 211) promote the application and interactive comparison analysis of data from different research groups. Also,