

DEVELOPMENT OF *DAPHNIA MAGNA* UNDER EXPOSURE TO AMPICILLIN

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Abstract

In this study, the chronic effects of Ampicillin on survival, reproduction and growth of *Daphnia magna* was monitored during 21 days exposure test. The results showed that Ampicillin strongly affected on life history traits of *Daphnia magna*, i.e. 47% reduction of survival proportion, lower 68% reproduction than the control, and reduction in body length and in the intrinsic population rate, especially at its highest concentration. The antibiotics especially Ampicillin should be included in the Vietnam guidelines for environmental and ecological protection.

Keywords: Ampicillin; Antibiotic; Chronic effects; *Daphnia magna*; Life history traits.

1. INTRODUCTION

In the last decades, there have been increasing concerns on the negative effects of pharmaceutical products to wildlife because they can include complex compounds which are toxic to aquatic organisms even at low concentrations [1, 13]. Antibiotics are among the most common pharmaceutical products which are most used in aquaculture and hospitals which can be transported directly into surface water or accumulate in the sediment [7, 18, 29]. Xu et al. (2007) has found that the concentration of some antibiotics in surface water of Victoria Harbor (Hong Kong) and the Pearl River (Guangzhou) were varied from 3.3 to 460 460 µg/L depending on location and season. While Khan et al. (2013) detected several antibiotics, such as

oxytetracycline, trimethoprim, and sulfamethoxazole in downstream of Indus River (Pakistan) with concentrations of 1100, 1700 and 2700 ng/L, respectively. According to Hussain et al. (2016), antibiotics from wastewater could accumulate in soil and plants, and percolate to groundwater. García-Galán et al. (2010) found a large variation on concentrations of sulfonamides class in groundwater at Catalonia, Spain, from 0.01–3460 ng/L. Whereas, Javid et al. (2016) found concentrations of Tetracycline from 5.4 to 8.1 ng/L in groundwater in Varamin Plain and Yaftabad, Iran.

In Vietnam, there have been around 138 antibiotics used in aquaculture, of which 32 antibiotics were used for shrimp culture and 39 for shrimp larvae rearing [14]. Pham et al. (2015) found some antibiotics such as

trimethoprim, oxytetracycline, sulfamethoxazole and sulfadiazine at shrimp culture. Additionally, the common antibiotics were detected in wastewater from hospitals and health care centers in Ho Chi Minh City and nearly 20% of effluent samples collected from the wastewater treatment plants containing antibiotics at the concentrations exceeded the Vietnam national standard limits [29].

Recently, there have been many studies on the effects of antibiotics on aquatic organisms, especially zooplankton. For example, Gorokhova et al. (2015) proved that antibiotics might acutely impact on non-target *D. magna* via changes in their microbiota. Consequently, decreased feeding and digestion were observed in the animals when they exposed to 0.25–2 mg/L of trimethoprim for 48 h. Additionally, di Delupis et al. (1992) investigated the acute toxicity of four antibiotics to *D. magna* and found the toxicity order of bacitracin > erythromycin > aminosidine > lincomycin. The author observed that after 72 h of exposure, lincomycin demonstrated its toxicity and caused a death rate of 85% at the concentration of 800 mg/L. Besides, at low concentrations (5–100 mg/L), lincomycin lowered the phototactic activity from 70% to 48%. In contrast, aminosidine at the concentrations of 10–500 mg/L increased the positive reaction towards light from 86–122%. erythromycin did not alter the positive phototaxis. Similarly, Macri et al. (1988) announced that furazolidone antibiotic caused highly impacts to *D. magna* but did not to *Artemia salina*.

Amongst antibiotics, ampicillin belongs to β -Lactams antibiotic and is one of the most widely used antibacterial drugs in veterinary medicine [24]. However, the toxicity of this compound to micro-crustacean, specially *D. magna* is not fully and clearly understood. Therefore, in this study, we investigated the chronic effects of ampicillin on the life history trait of the micro-crustacean *D. magna*.

2. MATERIALS AND METHODS

2.1. Experimental organisms and chemicals

The test organism was *Daphnia magna* Straus, purchased from MicroBio Tests Inc. Belgium. The animal has been fed with green alga *Chlorella* sp. and maintained in the laboratory conditions of $22 \pm 1^\circ\text{C}$, dim light and light dark cycle of 14h:10h, for many generations. The *D. magna* was reared in ISO medium consisted of NaHCO_3 , CaCl_2 , MgSO_4 and KCl while the green alga was cultivated in Z8 medium under continuous aeration [5].

Before to the experiment, thirty active adolescent female daphnids were selected and incubated in a 500 mL beaker containing 400 mL ISO medium for approximately 2–3 weeks. During this period, the daphnids were fed with ad libitum alga. The offspring (<24 h) from the second to third clutch of these female *Daphnia* were chosen randomly for the test.

The chemical ampicillin anhydrous $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ (99% from Sigma Aldrich, St. Louis, Mo., USA) was used for the chronic exposure to *D. magna*. Because ampicillin rapidly loses activity when stored above a pH of 7.0 [3, 10, 19], the antibiotic solution was dissolved in acetate buffers which pH value was approximately 4 [3, 10]. The concentration of this stock was 5 mg/L and stored at the temperature of 4°C .

2.2. Experimental setup

The neonates (15 individuals per treatment) were individually incubated in 50 mL glass cups containing 20 mL ISO medium solely considered as control. For the antibiotic exposures, *D. magna* was incubated in medium containing 5, 50 and 250 μg ampicillin/L (abbreviated as 5, 50 and 250 ppb). The animals were incubated in the laboratory conditions as mentioned above. The test media were totally renewed 3 times a week, and the animals were fed with *Chlorella* sp. at the concentration of 1 mg/L per d. All incubations lasted for 3 weeks. The fitness parameters of *Daphnia* daily observed were survival, maturity age (the time of the first egg appeared in the brood chamber of *Daphnia*), and reproduction. By the end of the test, all living *Daphnia* female were immediately fixed with Lugol solution [2], and body length was measured on the microscope (Olympus BX 51) coupled with a digital camera (DP71) to evaluate the growth of the animal. Besides, age-specific survival and clutch size were used to estimate the intrinsic rate of population increase, as a measure of fitness.

2.3. Data analyses

Signaplot version 12.0 was used for the data processing. Kruskal-Wallis test was applied for calculation on the statistically significant difference of the maturation, the fecundity and the intrinsic growth rate of *D. magna*. The Euler equation [28] was used to calculate the intrinsic rate of population increase:

$$(r): 1 = \sum e^{-rx} l_x m_x$$

Where:

x – age (in days)

l_x – the probability of surviving

m_x – the fecundity at age x .

3. RESULTS AND DISCUSSION

3.1. Effects on survival

As seen in Fig. 1, at the end of this experiment, the survival of *D. magna* in the control treatment reduced to 93% that was within the eligible range of values of chronic toxicity test according to Rice and Bridgewater (2012). Exposed to ampicillin at a concentration of 50 ppb, survival proportion of *D. magna* maintained at 100% during 18 days of exposure but became the same as the control treatment by the end of the experiment, day 21. However, survival of daphnids in 5 and 250 ppb exposures was gradually decreased to 67% and 47%, respectively, when the test terminated (Fig. 1). Our results were in line with the previous study of Dalla Bona et al. (2014) in which both enrofloxacin (at the concentration of 0.9 mg/L) and sulfaguanidine (at the concentration of 2.5 mg/L) reduced the survival of *D. magna* to less than 30%, whereas, ciprofloxacin (5 mg/L) and sulfaguanidine (3.2 mg/L) significantly reduced the survival of *Daphnia curvirostris* to less than 10% after 13 days of incubation. Besides, this result was in agreement with the investigation of Park and Choi (2008) revealing that the antibiotics of β -lactam class were toxic to *Vibrio fischeri*, *D. magna*, *Moina macrocopa*, and *Oryzias latipes*. Antibiotics could impact on bacteria-mediated digestion in *D. magna* leading to the limitation on nutrient breakdown and absorption in the animals [12], consequently energy cost. This may help to explain the mortality increase in ampicillin treatments in our study. Further investigations to confirm the negative influence of ampicillin to the useful bacterial communities in *Daphnia* digestive tract are suggested.

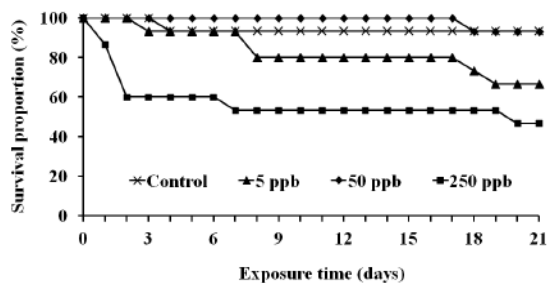


Figure 1. Survival of *Daphnia magna* chronically exposed to Ampicillin

3.2. Effects on maturation

The influence of ampicillin on the maturation of *D. magna* was showed in Fig. 2. *Daphnia magna* in 50 ppb treatment reached their maturation around the

age of 4 days old, significantly earlier than the control and other ampicillin exposures (5 ppb and 250 ppb; $p < 0.001$), which took around 6 days. The stimulation on maturation in 50 ppb treatment could be an adaptation of daphnids under the unfavorable conditions [22]. It is notable that Ampicillin in several specific concentrations was not highly toxic to the aquatic organism and algae comparing to the other antibiotics [27]. Moreover, the comparable concentration of ampicillin could be impellent the maturation of *D. magna* which was showed in our study. However, the stimulation of maturation, which was presented by toxic substances, was able to lead to the serious issues at the next generation such as the death and the malformation of the neonates [21].

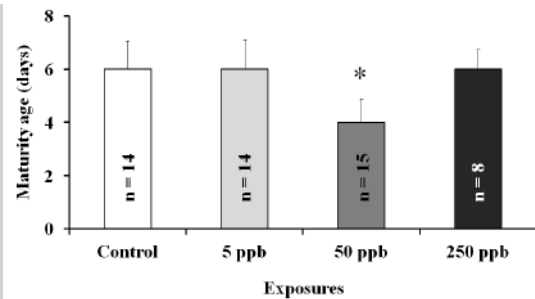


Figure 2. Maturity age of *Daphnia magna* (mean value \pm SD) chronically exposed to Ampicillin. Asterisk indicates significant difference between control and exposures by Kruskal-Wallis test (*, $P < 0.001$)

3.3. Effects on reproduction

In control, the clutch size of a mother *D. magna* was around 8 offsprings. However, the clutch size of *D. magna* was slightly increased in the 5 ppb and 50 ppb treatments. The average number of offspring per clutch in these treatments were approximately 9 neonates whereas the clutch size in 250 ppb treatment were decreased to 7 neonates (Table 1).

Table 1. Reproduction of *Daphnia magna* exposed to Ampicillin at several exposures in 21 days. Asterisk indicates significant difference between control and exposures by Kruskal-Wallis test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

Exposures	Total offspring	Number of broods per mother daphnid	Number of neonates per clutch
Control	602	5 \pm 1.5	8 \pm 2.94
5 ppb	452	3 \pm 1.8**	9 \pm 2.81*
50 ppb	783	6 \pm 1.0	9 \pm 3.66*
250 ppb	193	2 \pm 1.9***	7 \pm 3.57

In addition, during three weeks of experiment, the total accumulative offspring in control was 602 offsprings. However, in comparison with the number of neonates in control, that in the 5 ppb and 250 ppb treatments decreased considerably to 75% and 32%, respectively. Regarding to brood number per female, there were a strong reduction from approximately 5 broods in control to around 3 and 2 broods in 5 ppb and 250 ppb treatment, respectively. Especially, because experimental organisms exposed 50 ppb ampicillin reached early maturity day, the total neonate and the brood number per female in this treatment were highest, 783 offsprings and around 6 broods. These results were in consistent with a previous study of Giordano et al. (2010) who discovered that ampicillin at high concentration reduced egg number of an invertebrate species, *Folsomia candida*.

3.4. Effects on body length

At the end of the experiment, the average body length of female *D. magna* in the control was around 3.42 mm. In 5, 50, and 250 ppb ampicillin exposures, those decreased to 3.38, 3.37, and 2.98 mm, respectively (Fig. 3). The inhibition of growth or shorten body length in the 250 ppb exposure in this study was in line with a previous investigation in which ampicillin caused a significant decrease of *Folsomia candida*'s body length [11].

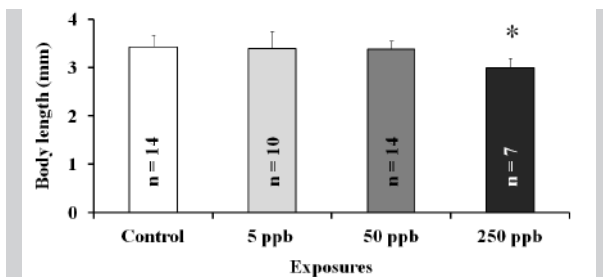


Figure 3. Growth of *Daphnia magna* (mean value \pm SD) chronically exposed to Ampicillin. The asterisk indicated significant difference between control and exposures by Kruskal-Wallis test (*, $P < 0.001$).

3.5. Effects on intrinsic growth rate

As mentioned above, the ampicillin exposures strongly affected on survivor, maturity and reproduction of animals, consequently those effects on the intrinsic population rate. *Daphnia* in 5 ppb and 250 ppb treatments had a significantly lower intrinsic rate of population than that of the control experiments. The intrinsic population rate of *Daphnia* in control was

0.333 whereas those in 5 ppb and 250 ppb ampicillin treatments were comparable, 0.315 and 0.296, respectively (Fig. 4). On the other hand, the intrinsic growth rate of the daphnids in 50 ppb treatment was highest, reached 0.417 due to the earlier maturation of the daphnids. Ferrando et al. (1995) reported that population growth would be determined primarily by the frequency of the first few broods which was supported by our results (Table 1 and Fig. 4).

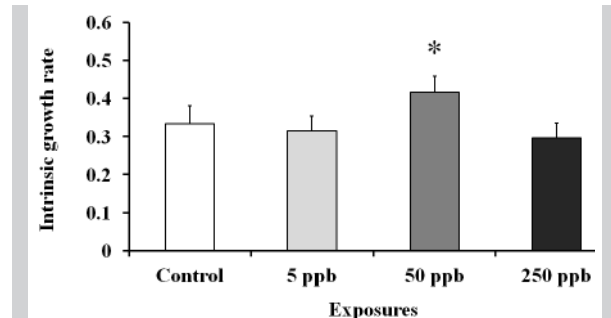


Figure 4. The intrinsic growth rate of *Daphnia magna* (mean value \pm SD) chronically exposed to ampicillin. Asterisk indicates significant difference between control and exposures by Kruskal-Wallis test (*, $P < 0.001$).

4. CONCLUSIONS

This investigation revealed that Ampicillin has detrimental impacts on the life history traits of *D. magna* including survival, maturation, growth and reproduction. We recommend further investigation including multiple generations of daphnids to antibiotics and antibiotic bio-accumulation should be conducted to fully assess the effects of antibiotics in general and Ampicillin in particular on micro-crustacean. Also, more attention should be paid to the presence, distribution, and fate of ampicillin in aquatic ecosystems.

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