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Residues of 2-hydroxy-3-phenylpyrazine, a degradation product of some β-lactam antibiotics, in environmental water in Vietnam

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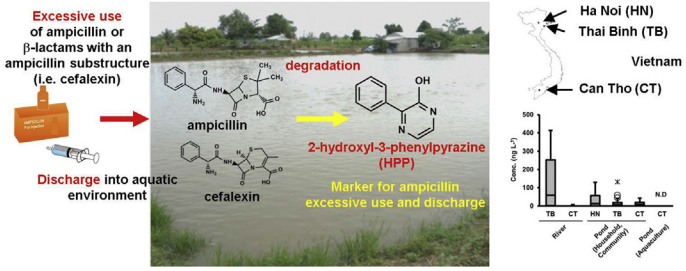
HIGHLIGHTS

- A method to detect 2-hydroxy-3-phenylpyrazine was validated with LC/MS/MS.
- HPP residues were detected in 61% of samples, ranging from 1.3 to 413.3 ng L^{-1}.
- Residue levels in rivers in city centres were higher than those in other sites.
- No sample from aquacultural ponds was found to contain HPP residue.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotic-resistant bacteria have become a serious problem worldwide, caused in part by the excessive use and discharge of antibiotics into the environment. Ampicillin (ABPC) is a widely used antibiotic. However, this chemical rapidly decomposes in water containing divalent cations like Ca^{2+} and Mg^{2+}, thus, detection of ABPC in environmental water is difficult. This study was carried out to evaluate the presence of 2-hydroxy-3-phenylpyrazine (HPP), one of the degradation products of ABPC and β-lactam antibiotics with an ABPC substructure, in environmental water. An analytical method for HPP monitoring in environmental water was developed using liquid chromatography/tandem mass spectrometry. The analyte was extracted from water samples and enriched using a solid-phase extraction cartridge. The quantification limit was 1 ng L^{-1}. The HPP recovery rates from spiked water samples of 25 and 125 ng L^{-1} were 84.1 and 86.1%, respectively. The method was then used to determine HPP residue levels in 98 environmental water samples from rivers, household ponds, and aquacultural ponds in Vietnam. HPP residues were detected in 60 samples. The HPP detection rates in rivers and household ponds were 42 and 79%, respectively. HPP was not detected in aquacultural ponds. HPP residue concentrations in the samples ranged from 1.3 to 413.3 ng L^{-1}. The residue levels in rivers flowing through city centres were higher than levels in other sampling locations. The findings of this study suggest that HPP is a promising

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marker for assessing the discharge of ABPC and β-lactam antibiotics with an ABPC substructure into the environment around sampling sites.

1. Introduction

In recent years, antibiotic residues have been found in aquatic environments in many countries, including in Europe, North America, and Asia (Kim and Carlson, 2007; Rosal et al., 2010; Li et al., 2012). Antibiotics enter the environment via various pathways, including wastewater effluent and runoff from land containing agricultural or human waste discharge. Antibiotic residues in the environment have been found at low levels, usually in the ng L^{-1}–μg L^{-1} range (Hoa et al., 2011; Li et al., 2012; Hon et al., 2016). Although such levels do not inhibit bacterial growth, some studies have suggested that they may contribute to the occurrence of antibiotic-resistant bacteria (ARB) through various mechanisms, including by inducing DNA mutation (Gullberg et al., 2011). Therefore, since the aquatic environment is a potential reservoir of ARB (Hoa et al., 2008; Kim et al., 2012; Bogialli et al., 2004), it is important to monitor antibiotic residues in environmental water in an effort to identify the mechanisms behind ARB prevalence and devise strategies to prevent the spread of ARB.

Vietnam has been growing rapidly, with an average GDP growth rate of 6.4 percent annually from 1985 to 2015 (The World Bank, 2016). The consumption of medications, including antibiotics, by both humans and livestock has also increased (Kim et al., 2013; Carrique-Mas et al., 2015; Gelband et al., 2015; Van Boeckel et al., 2015). Especially, ampicillin (ABPC), a penicillin-type antibiotic, is widely used because of its broad-spectrum applicability and low cost. We had previously determined that ABPC is very popular and sold frequently by performing an interview study of residents and farmers in the Ha Noi and Thai Binh provinces in Vietnam combined with a review of pharmacy sales records there (Sumimura et al., unpublished data). Such high usage of ABPC seems to cause the spread of antibiotic resistant bacteria. In fact, more than half of all healthy human residents of the above mentioned areas were found to carry extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (Nakayama et al., 2015; Ueda et al., 2015). However, ABPC was not detected in any environmental water samples collected around the above-mentioned sites, although antibiotics including tetracyclines, sulfonamides, quinolones, and trimethoprim were detected (Data not shown). Moreover, other research groups (Le and Munekage, 2004; Masey et al., 2010; Hoa et al., 2011), as well as our own (Hon et al., 2016), frequently detected these antibiotics, but not ABPC, at other sites in Vietnam. Therefore, we would like to obtain evidence of ABPC discharge into the environment.

Since ABPC readily decomposes in water containing divalent cations such as Ca^{2+} and Mg^{2+} (Gozlan et al., 2013; Mitchell et al., 2014), detection in environmental water is difficult. ABPC forms degradation products that include ampicilloic acid, ampilloic acid, ampicillin diketopiperazine, and 2-hydroxy-3-phenylpyrazine (HPP) (Fig. 1). Although several studies have investigated ABPC degradation products in non-environmental laboratory conditions in recent years (Garcia et al., 1998; Chung et al., 2009; Elmolla and Chaudhuri, 2010), the study of ABPC degradation products in actual environmental conditions has been limited. We can also hypothesize that the same degradation products of ABPC are generated from other β-lactams that have an ABPC substructure. However, the presence of such degradation products in environmental water would offer evidence that some β-lactam antibiotics including ABPC discharge into environment, which would cause the spread of β-lactam resistant bacteria.

In this study, we demonstrated that HPP is the stable degradation product of ABPC. Thus, we developed an analytical method to monitor HPP in environmental water and surveyed residual HPP levels at various sites in Vietnam. To the best of our knowledge, no previous study has reported on HPP levels in environmental water.

2. Materials and methods

2.1. Chemicals

Liquid chromatography/mass spectrometry (LC/MS)-grade acetonitrile, formic acid, and pure water were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) for liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. High-performance liquid chromatography (HPLC)-grade methanol and pure water were purchased from Merck Millipore (Massachusetts, USA) for solid phase extraction (SPE). Hydrochloric acid (guaranteed reagent) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). An analytical standard of ABPC for HPLC was obtained from Wako Pure Chemical Industries Ltd. and an HPP standard was purchased from Toronto Research Chemicals (Toronto, Canada).

2.2. Study sites and environmental sampling

This study was conducted in two regions of Vietnam: the Red River Delta in northern Vietnam and the Mekong River Delta in southern Vietnam. Within the Red River Delta, Ha Noi and Thai Binh were chosen as sampling locations. Within the Mekong River Delta, Can Tho was chosen (Fig. 2). Water samples were collected from one location in each pond and river in the urban and rural areas of each city. The number of samples from each site is presented in Table 1. From each sample, a volume of 500 mL was put into a plastic bottle and immediately transferred to the nearest laboratory (National Institute of Nutrition, Ha Noi; Thai Binh University of Medicine and Pharmacy; or Can Tho University). We had previously confirmed that HPP was not absorbed into the plastic bottles, and that this did not affect our results.

2.3. Sample preparation

A volume of 500 mL of each sample was filtered through defatted cotton to remove suspended solids prior to pretreatment and sample preparation. Sample clean-up was performed on an HLB cartridge (6 cc, 500 mg, Waters). The cartridges were conditioned prior to sample loading with 6 mL of methanol followed by 10 mL of distilled water with an adjusted pH of 3.0. Each sample was then passed through the cartridge at a flow rate of 10–15 mL min^{-1} via a valve and suction pump. Afterward, the cartridges were rinsed with 100 mL of distilled water with an adjusted pH of 3.0, and water remaining inside the cartridges was flushed out with a plastic syringe. The cartridges with absorbed analytes were wrapped in aluminium foil. The temperature of the cartridges was maintained at 4 °C as they were transported to our