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Ubiquitous occurrence of sulfonamides in tropical Asian waters

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HIGHLIGHTS

- Sulfonamides, especially sulfamethoxazole, was dominant in sewage in tropical Asia.
- Sulfamethazine and oxytetracycline were dominant in livestock and aquaculture waste.
- ~10% of sulfamethoxazole in Mekong River was derived from pig-farm wastewater.
- 12 tons/year of sulfamethoxazole is supplied from Mekong River to South China Sea.

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ABSTRACT

Seven sulfonamides, trimethoprim, five macrolides, lincomycin and three tetracyclines were measured in 150 water samples of sewage, livestock and aquaculture wastewater, and river and coastal waters, in five tropical Asian countries. The sum of the concentrations of the target antibiotics in sewage and heavily sewage-impacted waters were at sub- to low-ppb levels. The most abundant antibiotic was sulfamethoxazole (SMX), followed by lincomycin and sulfathiazole. The average concentration of SMX in sewage or heavily sewage-impacted waters was 1720 ng/L in Vietnam (Hanoi, Ho Chi Minh, Can Tho; n = 15), 802 ng/L in the Philippines (Manila; n = 4), 538 ng/L in India (Kolkata; n = 4), 282 ng/L in Indonesia (Jakarta; n = 10), and 76 ng/L in Malaysia (Kuala Lumpur; n = 6). These concentrations were higher than those in Japan, China, Europe, the US and Canada. A predominance of sulfonamides, especially SMX, is notable in these tropical countries. The higher average concentrations, and the predominance of SMX, can be ascribed to the lower cost of the antibiotics. Both the concentration and composition of antibiotics in livestock and aquaculture wastewater varied widely. In many cases, sulfamethazine (SMT), oxytetracycline (OTC), lincomycin, and SMX were predominant in livestock and aquaculture wastewater. Both human and animal antibiotics were widely distributed in the respective receiving waters (i.e., the Mekong River and Manila Bay). SMT/SMX ratios indicate a significant contribution from livestock wastewater to the Mekong River and nearby canals, with an estimated ~10% of river water SMX derived from such wastewater. Mass flow calculations estimate that 12 tons of SMX is discharged annually from the Mekong River into the South China Sea. Riverine inputs of antibiotics may significantly increase the concentration of such antibiotics in the coastal waters.

1. Introduction

Human and veterinary antibiotics have been widely detected in municipal and agricultural wastewater and receiving waters (Kümmerer, 2009a). Antibiotics are biologically active, and their ecological impact has been a source of concern. Most notably, the emergence of antibiotics resistance is of great concern (Kümmerer, 2009b; Hoa et al., 2008). To assess the ecological impact of antibiotics in aquatic environments, it is essential to understand the types of antibiotics and their concentrations in the respective source (i.e., wastewater) and receiving water. There have been reports on environmental antibiotics in North America, Europe and East Asia (e.g., Hirsch et al., 1999; Miao et al., 2004; Göbel et al., 2005; Xu et al., 2007; Watkinson et al., 2007; Spongberg and Witter,

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2008; Lin et al., 2008; Li et al., 2009; Sim et al., 2011; García-Galán et al., 2011; Murata et al., 2011; Segura et al., 2009 and references therein), but these regions are located in cold and temperate climate zones, and very limited information is available on antibiotics in tropical, specifically South and Southeast Asian, waters (Managaki et al., 2007).

Infectious disease is more pervasive, and a higher percentage of people suffer from such disease, in tropical Asia than in North America, Europe or East Asia. According to WHO data (WHO, 2012), infectious and parasitic diseases account for 19% of total deaths in Southeast Asia, and only 3% in Europe and 5% in Americas. This Southeast Asian figure is probably due, in part, to the regional climate conditions (hot and wet), which are conducive to the incubation of vector microorganisms, and to insufficient sewer and water supply systems. The higher levels of infectious disease may lead to increased usage of antibiotics, and this, in turn, to higher concentrations of antibiotics in the tropical Asian waters. Due to economic factors, inexpensive antibiotics are frequently used in tropical Asian countries and could be detected widely in tropical Asian waters (Dang et al., 2011; Hoa et al., 2011; Takasu et al., 2011). More frequent exposure of bacteria to these antibiotics may develop and select antibiotic resistant bacteria to these antibiotics in the environment even at low concentrations (Knapp et al., 2008; Gullberg et al., 2011). There have, in fact, been several reports examining antibiotic-resistant genes in the tropical Asian aquatic environment (Agersø and Petersen, 2007; Hoa et al., 2008).

In addition to human medicine, large amounts of veterinary antibiotics are assumed to be used in the region, due to intensive husbandry and aquaculture activities. For example, integrated agricultural operations, such as Vietnam's common 'vegetable, aquaculture, caged-animal' (VAC) system, may present an increased risk of human exposure to antibiotics and antibiotic-resistant bacteria/genes (Suzuki and Hoa, 2012). Generally, wastewater treatment, though not totally effective by itself, reduces the environmental burden of antibiotics (Nakada et al., 2007). However, treatment of human waste (sewage) and animal waste is not, at present, adequately implemented in tropical Asian countries, as has been confirmed by the monitoring of molecular markers (Isobe et al., 2002, 2004). Furthermore, climate conditions (particularly, frequent heavy rain) may facilitate the transport of antibiotics to rivers and coastal zones, due to resulting overflows of sewage treatment systems (sewers, wastewater storage ponds, lagoons) and VAC systems, increased agricultural surface runoff, and rapid flushing through streams. In light of the foregoing, higher concentrations of antibiotics, especially inexpensive varieties, are a concern in tropical Asian countries. However, thus far, only very limited information (Managaki et al., 2007) has been available on the types, abundance, distribution and mass flow of antibiotics in tropical Asian waters.

In the present study, we measured seven sulfonamides, trimethoprim, five macrolides, lincomycin and three tetracyclines in sewage, canals heavily-impacted by sewage, wastewater from livestock farms and aquacultures, and rivers and coastal waters, in Vietnam, the Philippines, Indonesia, Malaysia and India. These antibiotics are the most commonly used worldwide.

2. Materials and methods

2.1. Study areas and samples

The sample details, including coordinates, dates, pH and electric conductivity, are listed in Tables A1, A2 and A3. Water samples were collected from urban drainage, canals and heavily sewage-impacted rivers in Vietnam (Hanoi, Ho Chi Minh City, Can Tho), the Philippines (Manila), Indonesia (Jakarta), India (Kolkata) and Malaysia (Kuala Lumpur), from 2006 to 2010 (Fig. 1). In the study areas, sewage treatment systems do not serve all residents and untreated sewage are directly discharged to canals and rivers. The percentage of the population served by sewage treatment ranged from 0% in Can Tho to 75% in Kuala Lumpur as indicated in Table A4. Livestock wastewater (pig-, cow-, chicken-farm wastewater and aquaculture wastewater) was collected in Vietnam (Hanoi, Ho Chi Minh City, Can Tho) and Thailand (Kohn Kaen). In Can Tho, water samples were collected from both rural and urban canals. River water samples were collected from the Mekong River, to investigate horizontal distribution, and diurnal and seasonal change, in antibiotic levels. In Manila, water samples were collected from Manila Bay, Laguna Bay and the Pasig River.

2.2. Analytical procedure

HPLC-grade solvent (water, methanol (MeOH), acetonitrile), ethylenediaminetetraacetic acid (EDTA; >99.5% purity), and formic acid (>99.5%) were supplied by Wako Pure Chemicals (Osaka, Japan). Sources, purity and acronyms of antibiotics standards, icluding isotopically-labeled antibitotics standards, are described in Table 1.

The samples were collected with a stainless steel bucket, stored in 1-L amber plastic bottles, transported cool to the laboratory, and filtered through pre-baked glass fiber filters (GF/F, Whatman). Antibiotics were analyzed according to Ye et al. (2006) with slight modifications. Antibiotics in the filtrates were extracted with 6-mL solid-phase extraction (SPE) cartridges (200 mg Oasis HLB resin, Waters). The cartridges were preconditioned with 6 mL methanol, 3 mL methanol containing 0.1% (v/v) formic acid, and 2 × 1.5 mL water. To adjust pH and prevent the chelation of tetracyclines with metal cations, EDTA was added as a solid and mixed, such that the EDTA concentration in the filtrate was 0.1%. Aliquots of the filtrate, with volumes ranging from 50 mL for sewage and animal wastes to 200 mL for river water as listed in Tables A5, 6 and 7, were passed through the conditioned SPE cartridges. After the extraction, water in SPE cartridges was removed by passing air for 30 s and the SPE cartridges containing the antibiotics were wrapped with aluminum foil and placed in a Ziploc bag. The cartridges were packed in dry ice during transport to the laboratory in Japan, then stored in a freezer at −30 °C until the time of analysis.

Just before analysis, each SPE cartridge was thawed and placed on a vacuum manifold. The cartridge was washed with 2 × 6 mL of water and dried in an air flow for 5 min. The analyte was then eluted with 4 × 2 mL methanol containing 0.1% (v/v) formic acid. The eluent was combined and spiked with an appropriate volume (50–200 μL) of an internal standard mixture consisting of sulfamethoxazole-d_4, clarithromycin-d_3, roxithromycin-d_9, and oxytetracycline-$^{13}C_1$, -d_3 (500 ppb each, in methanol). The eluent was then concentrated to around 0.5 mL in a rotary evaporator, and transferred to a 4 mL amber vial. The eluent in the vial was evaporated to complete dryness under a nitrogen stream at 80 °C, and dissolved in an appropriate volume (0.5 mL–40 mL) of H_2O/acetonitrile (94:6 v/v) containing 0.1% formic acid. The volume of the final solution is also listed in Tables A5, 6, and 7 to allow the calculation of the preconcentration factor, which ranged from 5 to 400. A 20-μL aliquot was injected into a liquid chromatograph (Agilent series 1100, Tokyo, Japan) equipped with a tandem mass spectrometer (TSQ Quantum 7000, Thermo Finnigan, Japan). The antibiotics quantified by mass spectrometer were separated in an Xterra MS C18 (2.1 mm i.d. × 50 mm; particle size: 2.5 μm; Waters) with a guard column (Xterra MS C18; 2.1 mm i.d. × 20 mm; particle size: 3.5 μm; Waters), using a binary gradient system (solvent A: 0.1% formic acid in H_2O; solvent B: acetonitrile), at a flow rate of 0.2 mL/min. The run started at 5% B for 5 min, followed by a 11-min linear gradient to 95% B, after which the initial conditions were reestablished and the column was equilibrated for 17 min. Analytes were quantified in selected reaction monitoring (SRM) mode with positive electrospray ionization (ESI) in positive mode. The operating conditions of the mass spectrometer are listed in Table A8. The *m/z* values of the precursur ion (Q1) and two monitored product ions (Q3) are listed in Table A9.

To identify antibiotics, we compared the retention times and the area ratios of the two product ions in each sample with the average retention time and peak ratios of standards in all measurements. The criteria difference between samples and the standard was within