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Occurrence and risk assessment of antibiotics in water and lettuce in Ghana

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HIGHLIGHTS

- Antibiotics occur in wastewater and lettuce samples in Ghana.
- All water bodies studied were significantly contaminated with antibiotics.
- Ciprofloxacin showed medium toxicity risk to algae.
- High risk for antibiotics resistance development in the environment could occur for ciprofloxacin, erythromycin, sulphamethoxazole and trimethoprim.
- Antibiotics loads were significantly reduced by WSPs up to 96%.

GRAPHICAL ABSTRACT

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ABSTRACT

Hospital wastewater and effluents from waste stabilization ponds in Kumasi, Ghana, are directly discharged as low quality water into nearby streams which are eventually used to irrigate vegetables. The presence of 12 commonly used antibiotics in Ghana (metronidazole, ciprofloxacin, erythromycin, trimethoprim, ampicillin, cefuroxime, sulfamethoxazole, amoxicillin, tetracycline, oxytetracycline, chlortetracycline and doxycycline) were investigated in water and lettuce samples collected in three different areas in Kumasi, Ghana. The water samples were from hospital wastewater, wastewater stabilization ponds, rivers and irrigation water, while the lettuce samples were from vegetable farms and market vendors. Antibiotics in water samples were extracted using SPE while antibiotics in lettuce samples were extracted using accelerated solvent extraction followed by SPE. All extracted antibiotics samples were analyzed by HPLC-MS/MS. All studied compounds were detected in concentrations significantly higher ($p = 0.01$) in hospital wastewater than in the other water sources. The highest concentration found in the present study was 15 μg/L for ciprofloxacin in hospital wastewater. Irrigation water samples analyzed had concentrations of antibiotics up to 0.2 μg/L. Wastewater stabilization ponds are low technology but effective means of removing antibiotics with removal efficiency up to 95% recorded in this study. However, some chemicals are still found in levels indicating medium to high risk of antibiotics resistance development in the environment. The total concentrations of antibiotics detected in edible lettuce tissues from vegetable farms and vegetable sellers at the markets were in the range of 12.0–104 and 11.0–41.4 ng/kg (fresh weight) respectively. The antibiotics found with high concentrations in all the samples were sulfamethoxazole, erythromycin, ciprofloxacin, cefuroxime and trimethoprim. Furthermore, our study confirms the presence of seven antibiotics in lettuce from irrigation farms and markets, suggesting an indirect exposure of humans to

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antibiotics through vegetable consumption and drinking water in Ghana. However, estimated daily intake for a standard 60 kg woman was 0.3 ng/day, indicating low risk for human health.

1. Introduction

Antibiotics are among contaminants of emerging concern. The main mode of entry of antibiotics into the aquatic environment is through wastewater (Karthikeyan and Meyer, 2006; Szekeres et al., 2017) and effluent from landfills (Eggen et al., 2010; Simon et al., 2005). Also, antibiotics are not completely removed in sewage treatment plants (Chang et al., 2010) and a wide range of antibiotics have been detected in wastewater stabilization ponds (WSPs) (Møller et al. 2016), municipal sewage (Castiglioni et al., 2006), hospital sewage (Duong et al., 2008), surface water (Alder et al., 2004; Arikan et al., 2008; Carmona et al., 2014; Kemper, 2008; Kolpin et al., 2002), and groundwater (Fram and Belitz, 2011; Hirsch et al., 1999; Hu et al., 2010; Lapworth et al., 2012).

The inherit effects of antibiotics include hypersensitive reactions, protracted toxic effects due to exposure to low-level antibiotics for a long time (Sarmah et al., 2006), antibiotic-resistant bacteria development and spread (van den Bogaard, 2000; Gullberg et al., 2011) and abnormalities in digestive system functioning (Bedford, 2000; Deitch, 1990; Schuijt et al., 2013). The public has easy accessibility to antibiotics in low and middle-income countries (LMICs), from a variety of sources, including drugstores, over-the-counter chemical shops, hospitals, and roadside stalls (Lerbech et al., 2014; Senah, 1997; Wolf-Gould et al., 1991). Antibiotics can sometimes be bought at pharmacies and drugstores in LMICs without prescription, despite prohibiting legislations to stop selling (Bekoe et al., 2014; Lerbech et al., 2014). Extensive accessibility to antibiotics in developing countries like Ghana could lead to continuous exposure to antibiotics via food and water. Studies have reported that 34–70% Ghanaians have their urine contaminated with antibiotics suggesting that the Ghanaian public may unintentionally be exposed to antibiotics (Lerbech et al., 2014). This is concerning, since studies indicate that safe levels of exposure may be extremely low, as low as 0.1 μg/L, due to the development of resistance at sub-minimum inhibitory concentration (sub-MIC) levels (Gullberg et al., 2011). Thus, studies are needed to identify antibiotics in the environment and potential human exposure via food and water.

The use of wastewater for irrigation is common in LMICs, including Ghana (Cornish and Aidoo, 2000; Drechsel and Keraita, 2014; Keraita et al., 2008). Usually, untreated and/or partially treated wastewater from urban areas of LMICs is discharged into drains, smaller streams and other tributaries of larger water bodies, where it is mixed with storm and freshwater (diluted wastewater) before it is used by farmers. This surface water is referred to as low quality water (Raschid-Sally and Jayakody, 2008).

Recent studies have demonstrated uptake of antibiotics by crop plants like lettuce and other vegetables under real or simulated field conditions (Ahmed et al., 2015; Azanu et al., 2016; Boxall et al., 2006; Chitescu et al., 2013; Christou et al., 2017; Dolliver et al., 2007; Riemenschneider et al., 2016) and their subsequent entrance to the food web via food consumption have been predicted (Azanu et al., 2016).

In Ghana, studies on the occurrence of hormones (Asem-Hiablie et al., 2013), pesticides (Adu-Kumi et al., 2010) and metals (Anim-Gyampo et al., 2013) in environmental samples have been extensively done but no research so far on antibiotics in the environment and no risk assessment on antibiotics in the environment have been conducted. Additionally, although antibiotics have been found in surface waters in some developing counties with similar wastewater management challenges like Ghana and uses low quality water for irrigation of vegetables, no study has recorded levels of antibiotics in vegetables irrigated with low quality water contaminated with antibiotics.

The purposes of this work were to study the occurrence and distribution of 12 antibiotics in hospital wastewater, rivers, WSPs wastewater, and low quality water used for vegetable irrigation. Furthermore, we performed a risk assessment on the antibiotics found in the water samples and estimated the risk quotients (RQs) for antibiotic resistance in various water bodies and estimated human exposure from lettuce irrigated with low quality water. Lettuce was chosen because it is cultivated all year round in Ghana, eaten with less or without cooking, and uptake studies performed using lettuce show that it could take up antibiotics (Azanu et al., 2016).

2. Materials and methods

2.1. Chemicals

A total of 12 antibiotics, all on the Ghana Essential Medicines List and the 2011 National Health Insurance Drug List (Ministry of Health, 2010; NHIA, 2011), were studied. These were ciprofloxacin (quinolone), erythromycin (macrolide), trimethoprim and sulfamethoxazole (sulfonamides), amoxicillin, ampicillin and cefuroxime (β-lactams), metronidazole (nitroimidazole) and doxycycline, tetracycline, chlorotetracycline and oxytetracycline (tetracyclines). The chemical structures and physicochemical properties are shown in Table 1. Selection of antibiotics was based on four factors: 1) frequency of prescribed usage for human-use in Ghana (Ministry of Health, 2004), 2) known or suspected environmental and species impact (Ash et al., 1999), 3) persistence in aquatic environments and previous detections in wastewater and surface waters (Kolpin et al., 2002), and 4) inclusion in previous studies of antibiotics in urine samples from outpatients in Ghana (Lerbech et al., 2014).

Amoxicillin (CAS #: 267-87-780, 98% pure) was obtained from Duchefa (Haarlem, Holland). Ampicillin (CAS #: 69-53-4, 97% pure), metronidazole (CAS #: 443-48-1, 98% pure), cefuroxime (CAS #: 55268-75-2, 97% pure), ciprofloxacin (CAS #: 85721-33-1, 96% pure), erythromycin (CAS #: 114-07-8, 97% pure), trimethoprim (CAS #: 738-70-5, 98% pure), and sulfamethoxazole (CAS #: 723-46-6, 97% pure) were purchased from Fluka (Brøndby, Denmark). Tetracycline hydrochloride (CAS #: 60-54-8, >96% pure), oxytetracycline (CAS #: 79-57-2, 97% pure) and chlorotetracycline (CAS #: 64-72-2, 97% pure) were purchased from Sigma-Aldrich (Steinheim, Germany), and doxycycline hydrochloride (CAS #: 564-25-0, 98% pure) was obtained from Takeda Pharma (Roskilde, Denmark). The internal standard (IS) d_4-sulfamethoxazole was purchased from Toronto Research Chemicals (Toronto, Canada), and the ISs d_8-ciprofloxacin and d_3-trimethoprim from QMX Laboratories (Thaxted, UK). Formic acid (98–100% pure, Ph Eur) was purchased from Merck KGaA (Darmstadt, Germany). Methanol (HPLC grade) was purchased from Lab-Scan (Gliwice, Poland). Antibiotics standard solutions were prepared by dissolving the solids in methanol and kept in freezer at −18 °C. Internal standard solution mixture and standard antibiotics-mix were with a concentration of 2.5 mg/mL and 5 mg/L respectively. These solutions were prepared by taken known amount of each stock solution and mixing with methanol in a 5 mL volumetric flask. The standard antibiotics-mix and internal standard solution-mix were kept in brown bottles to protect them from light and stored at −18 °C. The working standards for calibration were prepared few hours before analysis.