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Occurrence and ecological risk assessment of fluoroquinolone antibiotics in hospital waste of Lahore, Pakistan

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ABSTRACT

In the present study, wastewater and sludge samples of two major hospitals of Lahore, Pakistan were analyzed by developing an HPLC-UV method for the possible occurrence of five frequently used fluoroquinolone antibiotics i.e. ofloxacin, ciprofloxacin, sparfloxacin, moxifloxacin and gemifloxacin. The highest detected concentration was for moxifloxacin in both wastewater (224 μg/L) and sludge samples (219 μg/kg. The highest concentration of ofloxacin, ciprofloxacin, sparfloxacin and gemifloxacin were found to be 66, 18, 58 and 0.2 μg/L respectively. Risk quotient (RQ) was also calculated based on maximum measured concentrations and the RQ values were very high particularly for ofloxacin and ciprofloxacin. The maximum RQ values for ofloxacin against *Vibrio fisheri*, *Pseudomonas putida*, fish, *Daphnia*, Green algae and *Pseudokirchneriella subcapitata* were 3300, 66,000, 124, 46, 3300 and 6000, respectively. In case of ciprofloxacin, RQ values were found to be 1750 and 3500 against green algae and *Microcystis aeruginosa*, respectively.

1. Introduction

Pharmaceutical compounds (PCs) have emerged as potential contaminants for the total environment due to their extensive use and long term effects (Christen et al., 2010; Lombardo-Agüí et al., 2014). These PCs may interact with each other and can pose serious challenges to human healthcare. Such interactions of PCs may also lead to synergetic toxicity even at low concentration (Jones et al., 2005; Oliveira et al., 2015). Among PCs, antibiotics constitute a group that is widely used to treat several infectious diseases in both human and animals. These antibiotics have been found in almost all of the environmental matrices due to their excessive production, consumption and unsafe disposal (Rodriguez-Mozaz and Weinberg, 2010). These antibiotics accumulate in the environment matrices where they alter the local microbial ecology (Daughton and Ternes, 1999). Importantly, the presence of antibiotics in the environment has potentially induced higher level of antibiotic resistance among microbes than ever before. Recently, Teillant et al. (2015) have estimated that 30% decrease in antibiotic performance may cause 120,000 additional infections during surgery, and may lead to around 6300 deaths in USA. The frequency of infections and death toll may even rise in case of elevated antibiotic resistance. Liu et al. (2015) have isolated bacteria from patients and dairy animal in China that may even resist colistin. Colistin has been regarded as the 'drug of 21st century' that is used as 'last-line' therapeutic drug against multi drug resistant pathogens (Biswas et al., 2012). Recent evidences clearly warn against emerging antibiotic resistance levels, and global risk to human and animal health.

Hospital wastewater is a prominent source of PCs in the environment. Like other developing countries, the disposal of medical/hospital waste is not properly managed in Pakistan (Tudor et al., 2005). To the best of our knowledge, no hospital, irrespective of its size, has installed proper wastewater treatment facility in Pakistan. These hospitals discharge their wastewater directly into domestic wastewater channels. These channels carry wastewater to surface water channels. PCs present in hospital wastewater reach almost all of the environmental matrices. Only limited studies are available which provide the detail about the distribution of PCs in the environment of Pakistan. There is a likelihood that the concentration

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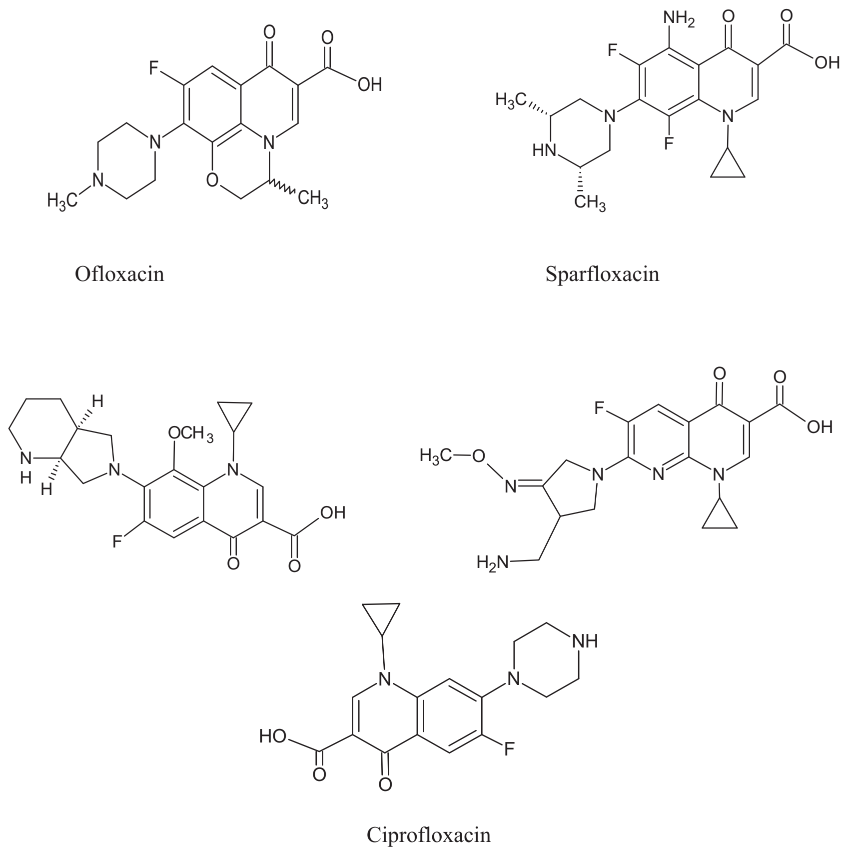

Fig. 1. Chemical structures of selected pharmaceuticals.

of PCs may be higher in Pakistan than other countries due to the absence of any baseline data about the monitoring of PCs and uncontrolled release of hospital wastewater. Thus, the development of new analytical methods is necessary to monitor PCs in the local environment (Rehman et al., 2015), especially based on available instruments like HPLC-UV due to financial constraints.

Quinolones and fluoroquinolones belong to the modern and more effective class of antibiotics used against respiratory diseases and bacterial infections (Turiel et al., 2005). They are particularly useful against both Gram-negative and Gram-positive bacterial infections (Alcaráz et al., 2014). Fluoroquinolones have been frequently detected at very high concentrations in different environmental matrices developed and developing countries due to their excessive use. High concentration of fluoroquinolones has already caused fluoroquinolone resistance in *Salmonella Typhi* and *Salmonella Paratyphi* in Pakistan (Hasan et al., 2008; Qamar et al., 2014). The monitoring of quinolones and fluoroquinolones in Pakistan is important due to high population density.

The aim of the present study was therefore to develop an HPLC-UV method using liquid-liquid extraction for the detection of five commonly used fluoroquinolones viz. ofloxacin, ciprofloxacin, sparfloxacin, moxifloxacin and gemifloxacin in the hospital wastewater of two major hospitals of Lahore, Pakistan. In addition, ecological risk assessment was also conducted against different types of species found in fresh water. Lahore is the second largest city of Pakistan and 14th largest city of the world with a population around 10 million. The chromatographic conditions were optimized for the detection of these pharmaceuticals in wastewater and sludge samples collected from the drains outside different wards of both hospitals. We hope the results of this study will establish a baseline data of antibiotics in the environment of Pakistan.

2. Experimental

2.1. Chemicals and reagents

Reference standards of fluoroquinolones were received from Schazoo Laboratories Pvt. Ltd., Lahore, Pakistan with claimed purity (ofloxacin = 99.75%, ciprofloxacin = 99.87%, sparfloxacin = 99.50%, moxifloxacin = 99.97% and gemifloxacin = 99.10%). The structures of the targeted pharmaceuticals are given in Fig. 1. HPLC grade methanol and analytical reagent grade triethylamine, potassium dihydrogen phosphate, phosphoric acid, *n*-hexane, ethyl acetate and chloroform were purchased from the local market and they were used without further purification. Double distilled water as well as all other solutions were filtered through 0.45 μm nylon filters (Millipore, USA) and degassed using ultrasonination before use.

2.2. Chromatographic conditions

LC-20A system (Shimadzu, Japan) was used for analytical method development and sample analysis. Samples were injected using auto sampler with loop having capacity of 20 μL. Ultraviolet detection was used for all the compounds at 279 nm. Separation was done using reversed phase discovery C18 column (250 mm × 4.6 mm, 5 μm particle size) at room temperature. Mobile phase was prepared by mixing methanol and 0.02 M phosphate buffer (pH 3.9) in the ratio of 40:60 (v/v). Phosphate buffer was prepared by adding 1 mL triethylamine in 1 L 0.02 M potassium dihydrogen phosphate and its pH was adjusted with phosphoric acid. All the experiments were performed isocratically at mobile phase flow rate of 1 mL/min.