Detection of antibiotics in hospital effluents in India

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Occurrence of antibiotics was investigated in water associated with two hospitals in Ujjain district, India. Samples of hospital associated water were subjected to solid phase extraction combined with high pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS), to estimate antibiotics in incoming safe water, hospital wastewater and groundwater. The incoming safe water and groundwater were free of antibiotics; however, metronidazole, norfloxacin, sulphamethoxazole, ceftriaxone, ofloxacin, ciprofloxacin, levofloxacin and tinidazole were detected in the range of 1.4–236.6 μg^{-1} in hospital effluents. Contamination of aquatic environment by antibiotic usage in hospitals has serious implications on public health and environment.

Keywords: Antibiotic residues, ceftriaxone, fluoroquinolones, hospital effluents, India.

THE estimates of global antibiotic consumption vary from 100,000 to 200,000 tonnes[1]. With passage of time, the problem of antibiotic resistance is increasing alarmingly. For example, in India, resistant bacteria have been reported in samples from diverse sources including hospital effluents[2–6]. The increasing problem of antibiotic resistance has repeatedly been placed on the global agenda as a threat to functioning health systems[7]. One of the sources of antibiotic resistance development is the presence of antibiotics in the environment, which generates foci of resistant bacteria through bacterial exposure to antibiotics[8]. The primary sources of antibiotic contamination of the environment are, waste from pharmaceutical plants (for example, in the Indian context work done by Larsson *et al.*[9]) and disposed unused antibiotics and excretion by humans and animals[8]. Much of the antibiotics used in humans and animals is not metabolized and a significant amount is added to the environment via urine and faeces, which gets mixed with receiving water[10]. Thus, hospital effluent is an important contributory source of antibiotics to the environment[11–13]. To understand the situation in India in this context, we undertook a case study regarding the antibiotic contamination levels in water associated with two hospitals in Ujjain district of Madhya Pradesh in India.

The two hospitals selected were: (1) the 350 bedded Ujjain Charitable Trust Hospital (UCTH) located at Ujjain and (2) the 500 bedded Chandrikaben Rashmikant Gardi Hospital (CRGH) located at Surasa. At these hospitals, samples were collected from (i) incoming safe water sources of the two hospitals, (ii) water exiting hospital building (hospital effluent) and (iii) groundwater sources in/near the hospital compounds. Sampling at one effluent exit point at both the hospitals was repeated within 4 h.

The method of sample collection, solid phase extraction (SPE) and analysis of antibiotics was based on Lindberg *et al.*[11]. Grab samples of 2 litres were collected from each sampling point, stored immediately at <4°C and were transferred to the analytical laboratory within 24 h. Samples were collected in screw capped amber bottles wrapped with silver foil to prevent photodegradation of fluoroquinolones and tetracyclines. At the laboratories of Shriram Institute for Industrial Research (SIIR) at Delhi, the samples were kept at –20°C until subjected to analysis. Thirteen antibiotics from different antibiotic groups (Table 1) were selected for chemical analysis based on the antibiotic prescription pattern in the inpatient wards of the two hospitals and also based on known and suspected environmental impact of various antibiotics[14].

For SPE, initially 50 ml of each sample was filtered through 0.45 micron and acidified to pH 3.0 by adding sulphuric acid. Then the sample was subjected to SPE with flow rate of the SPE column at 8 drops per minute. Sample was passed through activated C-18 cartridge activated with 5 ml of methanol, 5 ml methanol/water 50/50 (v/v) and 5 ml of water at pH 3.0. The cartridge was washed further with 5 ml of acidified water at pH 3.0. Then the cartridge was eluted with 5 ml of triethylamine (5% v/v) in methanol. The eluted solution was evaporated to 20 μl using nitrogen gas at 50°C. Finally, sample volume was reconstituted to 1 ml by adding water/acetonitrile 95/5 (v/v) and the required portion was injected in the LC-MS/MS system. Quantification of antibiotics was performed on 2695 series Alliance Quaternary Liquid Chromatographic system (Waters, USA) coupled with a triple Quadrapole Mass Spectrometer, Quatermicro API (Micromass, UK) equipped with an electro-spray interphase and mass lynx 4.1 software (Micromass, UK) for data acquisition and processing. All solvents and chemicals used were HPLC grade. Reference compounds and standards (98% purity) were purchased from Sigma, USP and Fluka.

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Table 1. Concentrations of various antibiotics in water samples from two hospitals in Ujjain district in India (sampling date 9 June 2007)

Type of water	Sample source	MET	TIN	SMX	ERY	NOR	CIP	OFL	LEV	DXY	AMI	CEF	AMX	CEFT
Ujjain Charitable Trust Hospital (UCTH) (antibiotic amount (μg^{-1}))														
Incoming water	Municipal water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Borewell	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hospital effluent	Drain 1	2.5	13.6	5.7	0.0	5.7	31.0	66.0	70.7	0.0	0.0	0.0	0.0	59.5
	Drain 2	0.0	0.0	2.7	0.0	0.0	8.0	2.7	3.3	0.0	0.0	0.0	0.0	58.3
	Drain 2 (repeat sample)	0.0	0.0	0.0	0.0	0.0	7.6	73.2	80.5	0.0	0.0	0.0	0.0	0.0
Groundwater proximate to hospital	Borewell	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chandrikaben Rashmikant Gardi Hospital (CRGH) (antibiotic amount (μg^{-1}))														
Incoming water	Borewell	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Municipal water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hospital effluent	Drain 1	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Drain 2	3.8	50.4	0.0	0.0	0.0	64.8	1.5	1.6	0.0	0.0	0.0	0.0	0.0
	Drain 3	1.4	88.4	81.1	0.0	20.6	67.3	5.6	6.8	0.0	0.0	0.0	0.0	0.0
	Drain 3 (repeat sample)	0.0	30.4	36.7	0.0	22.8	236.6	7.5	0.0	0.0	0.0	0.0	0.0	0.0
Groundwater proximate to hospital	Borewell	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

MET, Metronidazole; TIN, Tinidazole; SMX, Sulphamethoxazole; ERY, Erythromycin; NOR, Norfloxacin; CIP, Ciprofloxacin; OFL, Ofloxacin; LEV, Levofloxacin; DXY, Doxycycline; AMI, Amikacin; CEF, Cefoperazone; AMX, Amoxicillin, and CEFT, Ceftriaxone.

No antibiotics were detected either in incoming safe water or groundwater sources (borewell and hand pump) located in and around the hospital compound (Table 1). Eight of the 13 targeted antibiotics were detected in the analysed wastewater samples. The detected antibiotics were from the following groups; third generation cephalosporins, fluoroquinolones, sulphonamides and imidazole derivatives. In both the hospitals, fluoroquinolones were released in largest quantity followed by ceftriaxone at UCTH and tinidazole and sulphamethoxazole at CRGH. Ciprofloxacin was the most commonly detected antibiotic and was present in all effluent samples from both the hospitals.

High concentration of ciprofloxacin (up to 31,000 μg^{-1}) and also of other antibiotics was earlier reported from India, in the effluent from wastewater treatment plant serving drug manufacturers in south central India[9]. To our knowledge, our study is the first in India to have attempted quantitative estimation of antibiotics in hospital-associated water. The presence of different groups of antibiotics in hospital effluents in this study generally followed patterns of their reported stability and biodegradability in the environment[14].

The reason for non-detection of doxycycline in our study may be due to its high metabolic rate, which must have resulted in its absence in excretions and therefore in wastewater[14]. Similarly, the lack of the presence of unstable β-lactams as penicillin and other β-lactam based compounds is probably because they degrade quickly owing to their high susceptibility to hydrolysis. It is generally difficult therefore, to detect them in aquatic environment[15].

Imidazole derivatives (metronidazole and tinidazole) and sulphamethoxazole have low biodegradability in the aquatic environment[14] and similar to our study, they have been detected in hospital related water in other countries[11,12]. In the present study, high concentration of ceftriaxone was reported from two samples from UCTH (58.3 and 59.5 μg^{-1}). To our knowledge, this is the first report of the occurrence of ceftriaxone in hospital effluents. Fluoroquinolones were detected in all wastewater samples and their concentration was similar or higher compared to that found in hospital sewage studies elsewhere[11,12,16,17]. High concentration of fluoroquinolones in the aquatic environment can impact genotoxic effects. Modification in a strain of the bacteria *Salmonella typhimurium* has been reported at a concentration as low as 5 μg^{-1} for norfloxacin and 25 μg^{-1} for ciprofloxacin[16,18]. The reported concentration of both norfloxacin (20.6 μg^{-1}) and ciprofloxacin (236.6 μg^{-1}) was high in the present study in some samples. As wastewater treatment facilities are generally non-existent at hospitals in India, antibiotics may find their way into water sources and this is a matter of concern in terms of wider environmental and public health impact.

It is argued that antibiotic residues in aquatic environment are dilute compared to their therapeutic concentration and so may be harmless. However, exposure to concentrations below subtherapeutic level over long periods of time may be an ideal condition for selection and consequent spread of resistance[19], but not much data has been collected in this direction and this could be a focal point for future studies.

The variation detected in antibiotic concentration levels in repeat sampling at the same point could be expected because of variation in time and due to dynamics of excretion and water flow.

The comforting feature of the present study was the absence of antibiotic residues in municipal supplied drinking water and borewell water samples.

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Rainfall changes over tropical montane cloud forests of southern Western Ghats, India

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The southern Western Ghats tropical montane cloud forest sites (Gavi, Periyar, High wavys and Venniyar), which are characterized by frequent or seasonal cloud cover at the vegetation level, are considered one of the most threatened ecosystems in India and the world. Three out of four montane cloud forest sites studied in the southern Western Ghats had experienced diminishing trends of seasonal average and total rainfall, especially during summer monsoon season. The highest level of reduction for summer monsoon season was observed at Gavi rainforest station (>20 mm/14 years) in Kerala followed by Venniyar (>20 mm/20 years) site in Tamil Nadu. Average annual and total precipitation increased during the study period irrespective of the seasons over Periyar area, and the greatest values were recorded for season 2 (>25 mm/28 years). Positive trends for winter monsoon rainfall has been observed for three stations (Periyar, High wavys and Venniyar) except Gavi, and the trend was positive and significant (90%) for Periyar and High wavys. Increase in summer monsoon rainfall was observed for Periyar site and the trend was found to be significant (95%).

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TROPICAL montane cloud forests (TMCFs) occur where mountains are frequently enveloped by trade wind-derived orographic clouds and mist in combination with convective rainfall[1]. One of the most important direct impacts of frequent cloud cover is the deposition of cloud droplets through soil and vegetation surfaces (horizontal precipitation) which has immense hydrological importance in the cloud forest ecosystems[2]. Data collected from around the world show that horizontal precipitation can account for up to 14–18% and 15–100% of total precipitation during the wet and dry season respectively. A high annual deforestation rate in tropical mountain forests caused by harvesting fuel wood, resource logging and agricultural conversion is increasingly threatening cloud forest worldwide[3,4]. Although cloud forests provide habitats to many of the endangered species, most TMCFs are in the mountain ranges as the core of tropical biodiversity 'hotspots' that occupy approximately 0.4% of Earth's land surface while supporting about 20% and 16% of Earth's plants and vertebrates correspondingly[5]. Therefore, the conversion status of these unique ecosystems is critical and precarious as they are among the most endangered of all forest types[6].

India has only two TMCF mountain regions, which are located in north-eastern Indian states and southern Western Ghats bordering the states of Tamil Nadu and Kerala. Most of the southern Western Ghats seasonal montane cloud forests are located around the cardamom hills which are exposed to trade winds during the monsoon seasons. Among TMCF regions of the world, the Indian TMCFs are the least studied climatologically and explored biological wealth. Of all the types of tropical forest, tropical montane cloud forests are especially vulnerable to climate change[7]. The formation of cloud bank is affected not only by global climate change; there is also evidence that regional and local land-use change can have significant influence. Climatologists found that deforested Caribbean lowlands of Costa Rica remained