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Research Article

Occurrence of antibiotics in the selected hospitals and municipal wastewater system of Nepal

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Abstract

Hospital wastewater is contaminated with antibiotics excreted by inward patients and laboratory leftovers. This study aims to evaluate the occurrence of commonly prescribed antibiotics in two hospitals (Hospital 1 and Hospital 2) wastewater stream and Guheshwori municipal wastewater treatment plant by employing high performance liquid chromatography (HPLC). Identification of the most commonly prescribed antibiotics was done through questionnaire survey. For analysis, composite sampling was done following 3 days/24 hours mixing. HPLC (Agilent 1260 infinity II) system was used with DAD detector and binary pump. By matching the retention time with standards, samples (antibiotics) were identified, and the concentration of each identified antibiotic was determined by peak area calculation. Results indicate that amoxicillin, cefixime, ampicillin, ciprofloxacin, azithromycin, ceftriaxone, metronidazole and cloxacillin are the most commonly used antibiotics in Hospital 1 and Hospital 2. Study of physico-chemical parameters of the wastewater reveals the slightly alkaline pH, high electrical conductivity, high total dissolved solid and also high BOD & COD values. Interestingly, ceftriaxone and ciprofloxacin are detected in the inlet and outlet samples of hospital wastewater, and their concentration was reduced in outlet samples. From this study, antibiotics, albeit in low concentration, are detected in hospital wastewater and thus antibiotic specific water treatment system is recommended.

***Keywords*:** Antibiotics, effluent, hospital wastewater, influent, municipal wastewater

Introduction

For the past decades, the occurrence of micropollutants including antibiotics along with other pharmaceuticals, personal care products, illicit drugs, steroid hormones, industrial chemicals and pesticides in the aquatic environment has become a worldwide issue of increasing environmental concern and has been repeatedly observed in aqueous environment (Azanu et al., 2021; Tarigan et al., 2025). These micropollutants consist of huge range of anthropogenic as well as natural substances. Their presence is of concern due to the possible ecological impact (e.g., endocrine disruption) on biota within the environment. Of all groups of micropollutants, the vast majority of research activities are currently focused on the fate of active pharmaceutical ingredients during wastewater treatments (Polar, 2007; Giri, 2021). The important sub classes of pharmaceuticals are non-steroidal anti-inflammatory drugs (NSAIDs), lipid regulator, anticonvulsants, antibiotics, beta-blockers, and stimulants (Shaheen et al., 2022). Release of these pharmaceuticals to the environment is mainly via sewage outlets into surface water. They are usually found in very low concentrations (few ng/L to several g/L) in wastewater and surface water influenced by wastewater outlets. Nevertheless, chronic exposure of these pharmaceuticals to humans, flora and fauna even at these low concentrations is both of scientific and societal concern (Paut et al., 2022). The low concentration and diversity of micropollutants complicate the associated detection and analysis procedures and also create challenges for water and wastewater treatment processes (Luo et al., 2014).

The analysis of pharmaceuticals can be done by using high performance liquid chromatography coupled with mass spectroscopy (LC-MS) (Subedi et al., 2015) and also with high performance liquid chromatography with diode array UV detection (HPLC with UV-DAD detector) (Benito-Pena et al., 2006; Asperger et al., 2009).

Some antibiotics and their harmful metabolites are excreted from the human body after metabolism either through stool, urine or other body fluids, which later is disposed of into the hospital and municipal waste management system. Most of the antibiotics used for human and veterinary purposes remain unmetabolized and thus a considerable amount is added to the environment via excretion and eventually reaches wastewater treatment systems (Diwan et al., 2010; Jaidumrong et al., 2016). Incomplete removal of pharmaceuticals in wastewater treatment systems can result in the introduction of these antibiotics into the aquatic environment (Subedi et al., 2013). Another way of adding up these antibiotics to the environment is from pharmaceutical industries, as a result of dumping of unused antibiotics (Rooklidge, 2004; Kummerer, 2009). There has been increasing evidence regarding the toxic effects of these leftover antibiotics, such as, disturbing the normal flora of the soil to contributing in antibiotic resistance, toxicity to aquatic flora and fauna and ultimately to public health which is a serious threat to

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humanity (Kummerer, 2009; Subedi et al., 2013; Polianciuc et al., 2020). For example, it is found that ciprofloxacin antibiotic which falls under fluoroquinilone inhibits the growth of freshwater cyanobacteria, microalgae and macrophyte while aquatic animals and plants as invertebrate and duckweed were suppressed by amoxicillin and sulfamethoxazole (Jaidumrong et al., 2016).

In addition, the overuse and huge discharge of antibiotics has led to the emergence, development and spread of antibiotic resistance genes which are classified as one of the threats to public health by the World Health Organization (Bouki et al., 2013; Deng et al., 2016). There is a paucity of data regarding the existence of such hidden threats, in a developing country like Nepal where antibiotics are used overwhelmingly, and where proper hospital/municipal wastewater management is still below standard. Unfortunately, hardly any published data can be found on occurrence and fate of antibiotics in water/wastewater of Nepal. Therefore, local and large-scale studies are urgently needed to fully assess the occurrence of antibiotics in water/wastewater of Nepal. To reduce the hazards caused by antibiotics in the environment, assessment of antibiotics in wastewater can be an initiation to know its quantity so that the treatment system can be designed accordingly to meet the threshold value.

The overarching hypothesis of the current study is that the selected antibiotics persist in the wastewater system of the hospitals that might affect ecological system. The current study aims to (i) identify the most commonly prescribed antibiotics in the selected hospitals; (ii) assess their concentration in wastewater (if present) and (iii) evaluate the effectiveness of municipal and hospital wastewater treatment plants. Based on the results, we aim to propose the wastewater treatment plant systems that are specific to particular antibiotic/s.

Materials and Methods

Study Area and Sampling Site

The wastewater samples were collected from three sites, two hospital wastewater systems (Hospital 1 and Hospital 2) and one municipal wastewater treatment system (Guheshwori wastewater treatment system).

Hospital 1

It is a well-equipped hospital with 55 beds. On average, 55-60 patients visit the hospital on a daily basis and about 5-6 patients get admitted in a day. There is no proper wastewater treatment system in this hospital till date. Wastewater from this hospital is collected into a tank and discharged into the sewerage line. Samples were collected from the inlet and outlet of this tank.

Hospital 2

Hospital 2 is an independent, nonprofitable, non-government institution which is conceived and supported by the community. This hospital is equipped with 475 beds in total. This hospital has constructed a new decentralized wastewater treatment system (DEWATS) and it is functional from December 2019. Samples were collected from the inlet and outlet of the treatment system.

Guheshwori Wastewater Treatment Plant (WWTP)

It is the municipal wastewater treatment plant of Kathmandu. It is located at the bank of Bagmati River in Guheshwori, Kathmandu, the capital city of Nepal. This is the only operational municipal wastewater treatment system in Kathmandu where the treatment system is activated sludge type.

Wastewater Sample Collection

Wastewater sampling was done in October and November 2022 from Hospital 2 and 1 respectively. The wastewater sampling from municipal wastewater plant was done in April 2023. Wastewater samples were collected from inlet and outlet of hospital wastewater tank and municipal wastewater treatment plant. The samples were collected every 6 h in a day and all four samples were mixed to get 24 h composite. This was continued for three days and at the end, all the samples were again mixed, and a final 3 days/24 h composite sample was prepared. The samples were stored in black jar at 4°C during their transportation into the laboratory. The samples were then kept in refrigerator until sample pretreatment and analysis. The sample analysis was done with the help of HPLC to find out the occurrence and concentration of selected antibiotics in the wastewater samples from hospital and municipal wastewater treatment system.

Selection of Antibiotics

For the identification of the most common antibiotics in hospital wastewater, questionnaire survey was conducted with the doctors, the laboratory staff and the pharmacy staff of both the hospitals. Three different questionnaire surveys and few interviews were performed to find out the common antibiotics going into the wastewater stream of the hospitals. Ten doctors from ten different departments of Hospital 2 were approached to identify the most prescribed antibiotics for inward patients whereas doctors from 3 departments were available for survey at Hospital 1. Hospital laboratory staff were approached for the identification of different antibiotics used in their research activities and disposal mechanism of such antibiotics was also discussed. Regarding the disposal of the antibiotics used, staff from both the hospitals informed that the laboratory apparatus are washed in the sinks and autoclaving is done (where needed). Pharmacy of both the hospitals identified the most commonly sold antibiotics to inward patients. On the basis of the survey these antibiotics (ceftriaxone, amoxicillin, ampicillin, azithromycin, ciprofloxacin, amikacin) were selected for this study.

Wastewater Sample Analysis

The onsite parameters temperature, pH, conductivity and total dissolved solid (TDS) were analyzed by calibrated probes. Biological oxygen demand (BOD) and chemical oxygen demand (COD) were calculated respectively by 5-days BOD test and closed refluxcolorimetric method following standard methods (APHA, 2017).

For the assessment of antibiotics in wastewater, all the sample pretreatments (filtration and centrifugation) and sample analysis were done in ZEST Laboratories and Research Center Pvt. Ltd. (Bhaktapur, Nepal). The HPLC (Agilent 1260 infinity II) system was used with DAD detector and binary pump. Direct injections (without passing through the cartridge) of wastewater samples were done to the HPLC. Known concentration of solution of reference antibiotics was made in distilled water and then diluted as per need. The solvents used were acetonitrile:water at 60:40 ratios. The pH was maintained at 4.5. The limit of quantitation (LOQ) for the antibiotics were as follows: amikacin 6.91 ppm, ceftriaxone 0.133 ppm, amoxycillin 0.057 ppm, ampicillin 0.301 ppm, azithromycin 5.719 ppm and ciprofloxacin 0.055 ppm.

Results and Discussion

Onsite Parameters of Hospital Wastewater

The pH was found to be 7.44 ± 0.30 and 8.08 ± 0.38 respectively for Hospital 1 and 2 (Table 1) which is slightly alkaline. The electrical conductivity values were 1143.22 ± 77.10 and 1147.33 ± 98.86 for Hospital 1 and 2 respectively and total dissolved solids (TDS) values were respectively 333.78 ± 124.57 and 502.67 ± 134.81 for Hospital 1 and 2 (Table 1). These values seem to be higher, and it is obvious to get such higher value because wastewater contains lots of dissolved materials in it. TDS and conductivity findings suggest that there must be some salts and dissolved solids which contribute to increased TDS and conductivity.

Table 1. Onsite parameters of hospital wastewater

Parameters	Hospital 1 (Mean ± SD)	Hospital 2 (Mean ± SD
Temp (°C)	25.57 ± 0.37	20.63 ± 0.30
pH	7.44 ± 0.30	8.08 ± 0.38
Conductivity (µS/cm)	1143.22 ± 77.10	1147.33 ± 98.86
TDS (mg/L)	333.78 ± 124.57	502.67 ± 134.81

Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of Hospital Wastewaters

The wastewater samples collected from both the hospitals were analyzed for biological oxygen demand (BOD) and chemical oxygen demand (COD) analysis. The result (Table 2) showed that the COD of samples from both the hospitals was high, indicating existence of chemical pollutants in hospital wastewater stream. For wastewater, effluent water quality standard by Government of Nepal is 50 mg/L for COD and 250 mg/L for COD (MoE, 2010). Obtained value of BOD is very high (Table 2) showing the need of wastewater treatment system.

Table 2. BOD and COD of hospital wastewaters

Parameter	Hospital 1		Hospital 2	
	Inlet	Outlet	Inlet	Outlet
BOD (mg/L)	299.2	170	199.6	190.5
COD (mg/L)	516	436	487	433

Most Commonly Used Antibiotics in Hospitals

The survey results showed that amoxicillin, cefixime, ampicillin, ciprofloxacin, azithromycin, ceftriaxone, metronidazole and cloxacillin are the most commonly used antibiotics in Hospital 1 (Fig. 1). Likewise, ceftriaxone, ciprofloxacin, amoxicillin, cloxacillin, ampicillin, azithromycin, cefixime and metronidazole have been identified as the mostly used antibiotics in Hospital 2 (Fig. 2). One of the studies conducted in Nepal highlight that ceftriaxone, metronidazole, amoxicillin, cefixime and azithromycin are the most commonly used antibiotics in the emergency department of a tertiary health care center in Nepal (Basnet et al., 2024) which also supports our findings.

Concentration of Different Antibiotics in Hospital Wastewater

The two antibiotics (ceftriaxone and ciprofloxacin) were detected in wastewater of Hospital 1 (Table 3). The concentration of ceftriaxone was found to be 8.724 ppm in the wastewater samples collected from the inlet of the tank and 4.891 ppm in the outlet samples, while the concentration of ciprofloxacin was found to be 0.185 ppm in the inlet samples and 0.071 ppm in the outlet samples (Table 3). The storage tank might have been assisting in the reduction of concentration of antibiotics. Other antibiotics were below detection limit in the wastewater samples of Hospital 1.

In the wastewater samples of Hospital 2, ceftriaxone was found to be 3.667 ppm in the wastewater samples collected from inlet of decentralized wastewater treatment system (DEWATS) and 1.536 ppm from the outlet of DEWATS (Table 3). The treatment system seems to be effective in reducing the concentration of this antibiotic.

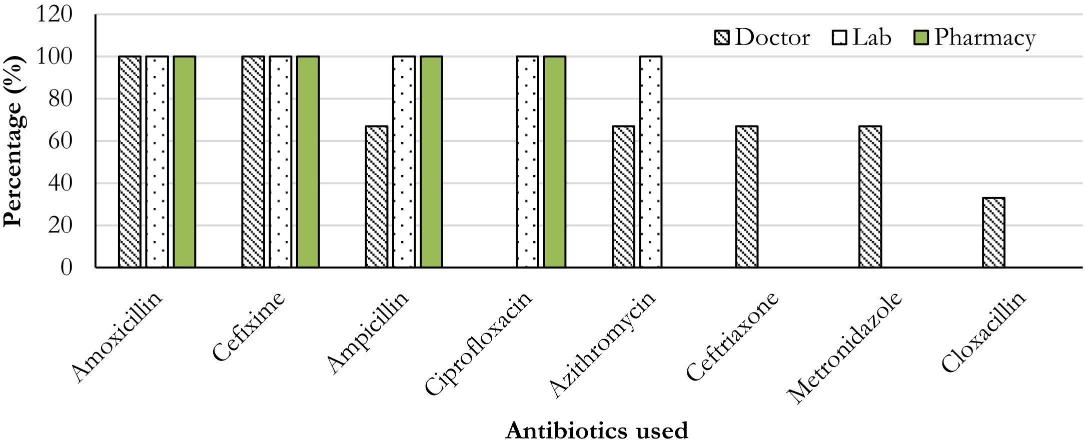

Figure 1. Commonly used antibiotics in Hospital 1

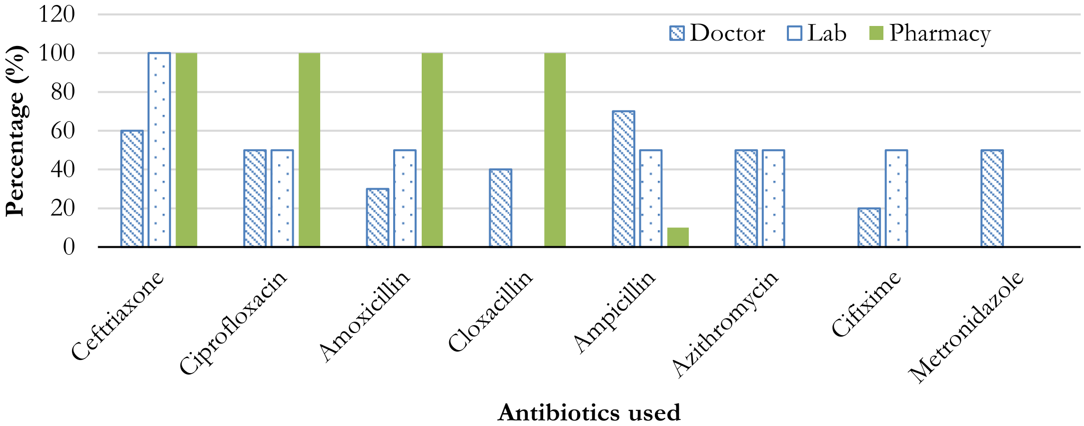

Figure 2. Commonly used antibiotics in Hospital 2

Table 3. Concentration of antibiotics in the hospital wastewater

Type of antibiotics	Concentration of antibiotics (ppm)			
	Hospital 1		Hospital 2	
	Inlet	**Outlet**	**Before treatment**	**After treatment**
Ceftriaxone	8.724	4.891	3.667	1.536
Amoxycillin	BDL	BDL	BDL	0.0815
Ampicillin	BDL	BDL	BDL	BDL
Azithromycin	BDL	BDL	BDL	BDL
Ciprofloxacin	0.185	0.071	BDL	BDL
Amikacin	BDL	BDL	BDL	8.385

(BDL=below detection limit)

From the questionnaire survey data, it was found that though ceftriaxone and ciprofloxacin were prescribed by doctors of both the hospitals, but ciprofloxacin is prescribed frequently by doctors from Hospital 1 (Fig 1 and 2). Similar result is found in hospital from India (Diwan et al., 2010). This might be the reason for showing ciprofloxacin concentration in wastewater of Hospital 1 only. Studies elsewhere also showed presence of ceftriaxone and ciprofloxacin in the hospital wastewater (Diwan et al., 2009; Lien et al., 2016; Aydin et al., 2019; Sinthuchai et al., 2021) but ciprofloxacin is detected in more numbers of hospital wastewater and has the higher concentration among antibiotics detected (Ebrahimi et al.,2020; Shokoohi et al., 2020). In addition, ceftriaxone is found to be showing lower removal efficiency of 0 – 62% (Chiemchaisri et al., 2022) than that of ciprofloxacin varying from 35- 93% (Ahmadzadeh et al., 2017).

In the wastewater of Hospital 2, amoxycillin (0.0815 ppm) and amikacin (8.385 ppm) were detected in the effluent wastewater samples only. These anomalous results are hard to explain as there is no possibility of detecting initially absent antibiotics in the outlet samples. It needs additional study to figure out if it is a noise peak or unwashed reference from the previous injection.

Concentration of Different Antibiotics in Municipal Wastewater

In the wastewater samples from Guheshwori wastewater treatment plant, all the studied antibiotics (ceftriaxone, amoxycillin, ampicillin, azithromycin, ciprofloxacin, and amikacin) were found to be below detection limit for both influent and effluent samples. This looks obvious and similar results of antibiotics concentration higher in hospital wastewater than in domestic wastewater are observed (Sinthuchai et al., 2021). The detection of antibiotics in municipal wastewater is a challenge due to low concentration and complex matrix of varieties of organic/inorganic deposits (Kosunen, 2015).

Methods for Removal of Antibiotics

When used in conjunction with traditional biological treatment, advanced wastewater treatment methods can greatly improve the removal of antibiotics prior to effluent disposal. In comparison to traditional procedures alone, modern treatment processes like advance oxidation process and different membrane bioreactors (MBRs) offer better effluent quality and more efficient removal of micropollutants, even if they may be more expensive (Barancheshme and Munir, 2019). Advance oxidation process includes ozonation, photo-fenton process, and heterogeneous photo catalytic process with a TiO_2 semiconductor while some of the membrane technologies are ultrafiltration, electrodialysis, nanofiltration, reverse osmosis (RO), membrane bioreactor (Karungamye et al., 2022).

In the context of Nepal, antibiotics mainly ceftriaxone and ciprofloxacin are present in the hospital wastewater system. Their presence can account for adverse effects in the nature and importantly it is relatively difficult to remove such antibiotics by traditional wastewater treatment plants. Antibiotic resistance, toxicity and significant effect on ecosystem (Karungamye et al., 2022) are some of the adverse effects that need immediate attention. Therefore, there is a need to design and develop treatment system which can remove or reduce the concentration of such antibiotics. Some major and useful techniques for the removal of ceftriaxone from aqueous systems include photochemical degradation, ion exchange, chemical oxidation, biological treatment, and adsorption (Badi et al., 2018). According to Hashemi et al., (2022), the performance of combined system $O_3/UV/Fe_3O_4@TiO_2$ was studied and revealed that there occurred 92.4% removal of ceftriaxone. Photocatalytic degradation seems to be effective in removal of ciprofloxacin, and the removal rate is found to be more than 80% (Lin et al., 2023). These are some of the techniques that could help in reducing the concentration of antibiotics that are detected herein.

Conclusions

This study shows that amoxicillin, cefixime, ampicillin, ciprofloxacin, azithromycin, ceftriaxone, metronidazole and cloxacillin are the most used antibiotics in Hospital 1 and Hospital 2. It is found that ceftriaxone and ciprofloxacin are detected in the hospital wastewater, and their concentration was reduced after storage and treatment. For the removal of ceftriaxone from aqueous systems, photochemical degradation, ion exchange, chemical oxidation, biological treatment, adsorption and also the combined system, e.g., $O_3/UV/Fe_3O_4@TiO_2$ can be performed. Similarly, photocatalytic degradation seems to be effective in removal of ciprofloxacin.

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Conflicts of Interest: The author declares no conflicts of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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