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Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam

Hong Anh Duong [a], Ngoc Ha Pham [a], Hoang Tung Nguyen [a], Thi Thuong Hoang [a], Hung Viet Pham [a], Van Ca Pham [b], Michael Berg [c], Walter Giger [c], Alfredo C. Alder [c,*]

[a] Centre for Environmental Technology and Sustainable Development, Hanoi University of Science, Vietnam National University, Hanoi, Nguyen Trai Road, Hanoi, Viet Nam
[b] National Institute for Control of Vaccines and Medical Biologicals, Ministry of Health, Dai Kim, Hanoi, Viet Nam
[c] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, 8600 Dübendorf, Switzerland

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ABSTRACT

Occurrence and behavior of fluoroquinolone antibacterial agents (FQs) were investigated in hospital wastewaters in Hanoi, Vietnam. Hospital wastewater in Hanoi is usually not treated and this untreated wastewater is directly discharged into one of the wastewater channels of the city and eventually reaches the ambient aquatic environment. The concentrations of the FQs, ciprofloxacin (CIP) and norfloxacin (NOR) in six hospital wastewaters ranged from 1.1 to 44 and from 0.9 to 17 $\mu g\,l^{-1}$, respectively. Total FQ loads to the city sewage system varied from 0.3 to 14 $g\,d^{-1}$. Additionally, the mass flows of CIP and NOR were investigated in the aqueous compartment in a small wastewater treatment facility of one hospital. The results showed that the FQ removal from the wastewater stream was between 80 and 85%, probably due to sorption on sewage sludge. Simultaneously, the numbers of *Escherichia coli* (*E. coli*) were measured and their resistance against CIP and NOR was evaluated by determining the minimum inhibitory concentration. Biological treatment lead to a 100-fold reduction in the number of *E. coli* but still more than a thousand *E. coli* colonies per 100 ml of wastewater effluent reached the receiving water. The highest resistance was found in *E. coli* strains of raw wastewater and the lowest in isolates of treated wastewater effluent. Thus, wastewater treatment is an efficient barrier to decrease the residual FQ levels and the number of resistant bacteria entering ambient waters. Due to the lack of municipal wastewater treatment plants, the onsite treatment of hospital wastewater before discharging into municipal sewers should be considered as a viable option and consequently implemented.

1. Introduction

The occurrence of antibacterial agents in the aquatic environment has led to an increasing concern about the potential environmental risks and the maintenance and spread of antibacterial resistance among microorganisms. We know almost nothing about the impacts of the environmental exposure to trace concentrations, but the detection of antibacterial resistant bacteria in wastewater discharges is worrying (Guardabassi et al., 1998; Schwartz et al., 2003; Chitnis et al., 2004; Volkmann et al., 2004). The global problem of antibacterial resistance is particularly urgent in developing countries where the infectious disease burden is high (Okeke et al., 2005). Resistance appears to have emerged and spread rapidly in many regions.

Hospital wastewaters are important sources of a large variety of pharmaceuticals including antibacterial agents, as evidenced by the fact that they occur in these wastewaters at higher concentrations than in wastewater from household effluents, which is due to high usage and low dilution. The environmental occurrence of pharmaceuticals has been widely reported and reviewed (Khetan and Collins, 2007). Concerning antimicrobial agents, several studies on their occurrence in wastewater and surface water have been published (Hirsch et al., 1999; Golet et al., 2002, 2003; Kolpin et al., 2002; Calamari et al., 2003; Giger et al., 2003; McArdell et al., 2003; Miao et al., 2004; Renew and Huang, 2004; Gobel et al., 2005, 2007; Joss et al., 2005; Lindberg et al., 2005, 2006; Nakata et al., 2005; Karthikeyan and Meyer, 2006; Mahnik et al., 2006; Batt et al., 2007; Gulkowska et al., 2008).

In the context of hospital wastewater discharge into the aquatic environment, the exposure of aquatic organisms to hazardous substances, particularly pharmaceuticals, disinfectants and radionuclides, should be considered (Emmanuel et al., 2005). Several studies have reported on the presence of antibacterials in hospital effluents (Guardabassi et al., 1998; Hartmann et al., 1998, 1999; Kummerer, 2001; Kummerer and Henninger, 2003; Loffler and Ternes, 2003; Ohlsen et al., 2003; Giger et al., 2003; Alder et al., 2004, 2006; Jarnheimer et al., 2004; Lindberg et al., 2004; Emmanuel et al., 2005; Brown et al., 2006).

* Corresponding author. Tel.: +41 44 823 54 78; fax: +41 44 823 53 11.
E-mail address: alfredo.alder@eawag.ch (A.C. Alder).

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Regulation in developing countries emphasizes limiting biological and chemical oxygen demand (BOD, COD) but not contaminants present in the treated effluents. Most of the studies on the occurrence of antimicrobials in the environment and related risk assessments have been performed in Europe and in North America. Relatively little is known about the situation in developing countries like Vietnam where the pharmaceutical market is rapidly growing and environmental regulations are not very well established. No quantitative data are yet available on the amounts of antimicrobials used in Vietnam. According to the number of registered brands and unofficial information from pharmacies and hospitals, β-lactams, macrolides and fluoroquinolones are the most widely used types.

In this study, we focused on fluoroquinolone antibacterial agents (FQs), including ciprofloxacin (CIP), norfloxacin (NOR), levofloxacin (LEV), ofloxacin (OFL) and lomefloxacin (LOME) because of their high consumption and their observed persistence in the aquatic environment. Very few wastewater treatment plants are in operation in Vietnam, and, therefore, contamination of surface waters in urban centres like Hanoi is a serious problem due to the discharge of untreated wastewater into the rivers, turning them into open sewers. Wastewater treatment basically occurs through self-remediation in small rivers, lakes and ponds in the city. Because hospital wastewaters are important point sources, they were chosen for this study to overview the environmental occurrence of FQs. Additionally, the fate of FQs was studied in a small hospital wastewater treatment facility where, as an exception, the wastewater of the hospital Huu Nghi is treated before it is discharged into the receiving ambient water.

To the best of our knowledge, this is the first investigation into the presence of residual concentrations of human-use antimicrobials in the aquatic environment in Vietnam. The results of this study are expected to be helpful to the Vietnam Environmental Protection Agency in assessing and minimizing the environmental impact of these emerging contaminants. In addition, this study aims to compare use patterns and occurrences of human-use antimicrobials in wastewater in Vietnam with the situation in European countries, taking into account that the use patterns and the wastewater treatment conditions vary significantly between different countries.

This study had three objectives: (1) to determine the occurrences of FQs in treated and untreated wastewater of some hospitals in Hanoi, which are of different size and have distinct departments and clinics, (2) to determine the removal efficiency in a small scale hospital wastewater treatment facility, (3) to present preliminary results of antibiotic resistance by determining the minimum inhibitory concentration (MIC) of FQs for *Escherichia coli* isolated from hospital wastewaters.

2. Experimental

2.1. Investigated hospitals and sample collection

Hanoi has a population of more than 3.5 million inhabitants (Duong et al., 2003). Most of the hospitals in the North of Vietnam are located in this area, including 18 polyclinic and 12 specialized hospitals. With a few exceptions no treatment of the hospital wastewater is performed and the untreated wastewater is directly discharged into the rivers flowing through Hanoi. The four main rivers To Lich, Lu, Set and Kim Nguu that run through the city from the north to the south are in reality open wastewater channels. The total municipal wastewater discharge of Hanoi is at present approximately 450 000 m^3 per day.

Six hospitals, including two polyclinic and four specialized hospitals with varying numbers of patients, were investigated. The information on medical specialization, number of patients, amount of wastewater and the existence of wastewater treatment for these hospitals is shown in Table 1. Hospitals consume significant quantities of water per day. The consumption in hospitals in industrialized countries varies from 0.4 to 1.2 m^3 per bed and day (Emmanuel et al., 2005), whereas in developing countries this consumption seems to be around 0.5 m^3 per bed and day (Laber et al., 1999). It should be mentioned that in Hanoi the number of beds and the number of patients staying overnight in the hospital are not equal, indicating demands beyond the hospital capacities.

The hospital Huu Nghi (Table 1) is a polyclinic hospital in which only high ranking officers are treated. In this hospital, a small wastewater treatment plant (WWTP) is operated. Thus, the Huu Nghi hospital was chosen to determine the mass flows of FQs in the aqueous compartment of this WWTP. Primary treatment of the wastewater entering the WWTP consists of a wastewater collection tank which acts as a primary clarifier and where the suspended solids are allowed to settle. The primary effluent is transferred into the activated sludge reactor and subsequently to the secondary clarifier. The combined hydraulic residence time in the primary clarifier, the aerobic reactor and the secondary clarifier was approximately 6.4 h, whereas the solid retention time was about 5–7 days. In an extraordinary setup, the secondary effluent passes afterwards through an anaerobic biological treatment system. After flowing through final laminar settling tank, the treated wastewater is discharged to the receiving water. A fraction of the secondary (return) sludge from the secondary clarifier is recycled to the activated sludge reactor and the excess sludge as well as the sludge from the anaerobic biological treatment is directed to the sludge collection tank. Water temperature at the sampling stations was between 15 and 20 °C. The suspended solids concentrations were 62 mg l^{-1} in the raw sewage, 48 mg l^{-1} in the effluent from the activated sludge reactor, 22 mg l^{-1} in the secondary effluent and 6 mg l^{-1} in the treated wastewater.

Grab samples of the wastewater were collected from five hospitals without wastewater treatment (Table 1: hospitals I–V) in August 2005, between 10 a.m. and 4 p.m. No composite samples could be collected because of the lack of sampling equipment.

At the treatment facility of the Huu Nghi hospital, wastewater was collected at four types of sample locations: (1) raw sewage, (2) effluent from the activated sludge reactor, (3) secondary effluent and (4) treated wastewater during two daily sampling campaigns. The first campaign was carried out during one day in September 2004 when 27 hourly grab samples and 4 composite samples were collected. In the second sampling campaign in April 2005, 40 grab samples were collected at each sampling site every 3 h and combined to four 24-h composite samples.

Table 1
Information on the investigated hospitals in Hanoi

Hospital	I Thanh Nhan	II Viet Duc	III Hanoi K	IV Central Obstetric	V Hanoi Obstetric	VI Huu Nghi
Type	Polyclinic	Surgery	Cancer	Obstetric, gynecology	Obstetric, gynecology	Polyclinic
Number of patients	500	1500	1200	700	630	400
Wastewater volume ($m^3\,d^{-1}$)	300	1000	300	335	230	300
Wastewater treatment	None	None	None	None	None	Yes
Daily wastewater volume per patient (m^3 per day and patient)	0.60	0.66	0.25	0.48	0.37	0.75

2.2. Chemical analysis and quality control

The methods applied to hospital wastewater, including material and reagents, were based on the procedure described by Golet et al. (2001). However, the sample volumes were reduced according to the wastewater matrix. Briefly, filtered (0.45 μm cellulose nitrate membrane filters) aqueous samples (20 ml) were concentrated by solid-phase extraction using mixed-phase cation exchange disk cartridges (MPC, Varian International) and subsequently measured by high-performance liquid chromatography with fluorescence detection. An internal standard procedure using the fluoroquinolone tosufloxacin (TOS) was used for quantification.

Breakthroughs were determined by extracting 20, 50, 70, 100 and 300 ml wastewater samples (duplicate analyses) using two stacked MPC cartridges. Wastewater samples where native FQs were not detectable were spiked with 400 ng of each FQ studied. The recoveries resulting from the first cartridge were 75–102%, 73–97%, 66–92%, 50–82%, 30–79%, 27–79% in 20, 50, 75, 100, 200 and 300 ml, respectively. Based on these results, 20 ml wastewater samples were chosen to provide quantitative extraction. The accuracy was assessed by recovery studies with MPC cartridges. Triplicate analysis was performed using 20 ml of municipal wastewater where native FQs were not detectable and spiked samples with 400 ng of each FQs and 4000 ng of TOS. The accuracy as indicated by the recovery ranged from 84% to 101%. The precision indicated by the relative standard deviation was lower than 10%. The limit of quantification (LOQ) in wastewater was defined as 10 times the signal to noise ratio (S/N $\geqslant$ 10). The corresponding LOQs for CIP, NOR, LEV/OFL and LOME were 0.05, 0.07, 0.07 and 0.08 $\mu g\,l^{-1}$, respectively. Blank samples were analyzed for each set of six samples to control for laboratory contamination and analytical interference.

2.3. Determination of the minimum inhibitory concentrations of CIP and NOR for E. coli isolated from wastewater of the Huu Nghi hospital

E. coli strains were first isolated from wastewater samples collected in several wastewater treatment steps at the Huu Nghi hospital. The *E*-test (AB Biodisk, Sweden) was conducted to determine the minimal inhibitory concentrations (MIC) of CIP and NOR for isolated *E. coli* on agar media. The *E*-test comprises of a plastic strip with a predefined gradient of antibiotic concentrations on one side, which is calibrated with an MIC reading scale in $\mu g\,ml^{-1}$ on the other side. When an *E*-test strip was applied to an inoculated agar plate, there was an immediate and effective release of the antibiotic with continuous and exponential gradient concentrations from the strip into the agar matrix. After incubation, during which bacterial growth becomes visible, a symmetrical inhibition ellipse centred along the strip is seen. The MIC value was read from the scale $\mu g\,ml^{-1}$ units where the ellipse edge intersects the strip.

3. Results and discussion

3.1. Concentrations and loads of FQs in hospital wastewater

In untreated hospital wastewater samples collected from six hospitals, only CIP and NOR were detected among the five FQs determined in this study. Concentrations and loads are listed in Table 2 together with data from the literature.

The other target FQs, including OFL and LEV, were not detected in any samples, although these FQs are highly consumed in European hospitals. In studies, in which the FQs were quantified by LC–MS, OFL was detected in Swedish and German hospital wastewaters at approximately the same levels as CIP (Ohlsen et al., 2003; Lindberg et al., 2004). Our results might be explained by the lower signal intensity of the co-eluting pair OFL/LEV at the emission wavelength used (445 nm) compared to CIP and NOR. In contrast to the method described by Golet et al. (2001), the emission wavelength was not changed to 500 nm to obtain maximum sensitivity for the pair OFL/LEV. Thus, a lower limit of quantification must be inferred.

CIP and NOR occurred in untreated hospital wastewaters in Hanoi at concentrations between 1.1 and 44 and from 0.9 to 17 $\mu g\,l^{-1}$, respectively. In four hospitals (II, III, V and VI, see Table 2), CIP was more abundant than NOR, while the opposite ratio was observed in the other two hospitals (I and IV). The CIP and NOR levels were

Table 2
Concentrations and loads of CIP and NOR in untreated wastewater effluents of hospitals in Hanoi and estimated FQ consumption per patient

Hospital	Concentration ± STD ($\mu g\,l^{-1}$)		Load[a] ± STD ($g\,d^{-1}$)			Estimated FQ consumption[b] (mg per day and patient^{-1})		Ref.
	CIP	NOR	CIP	NOR	Total FQs	CIP	NOR	
Hanoi, Vietnam								
I Thanh Nhan[c]	7.0 ± 0.1	15.2 ± 0.3	2.1 ± 0.0	4.6 ± 0.1	6.7 ± 0.1	7.6	30.7	This study
II Viet Duc[c]	10.9 ± 0.8	3.4 ± 0.4	10.9 ± 0.8	3.4 ± 0.4	14.3 ± 1.2	13.2	7.5	
III Hanoi K[c]	1.2 ± 0.2	<LOQ	0.4 ± 0.1	–	0.4 ± 0.1	0.5		
IV Central Obstetric[c]	2.1 ± 0.1	13.6 ± 0.3	0.7 ± 0.0	4.6 ± 0.1	5.3 ± 0.1	1.8	21.8	
V Hanoi Obstetric[c]	1.1 ± 0.1	<LOQ	0.3 ± 0.0	–	0.3 ± 0.0	0.9		
VI Huu Nghi[d]								
Raw wastewater	25.8 ± 8.1	8.4 ± 2.5	7.7 ± 2.4	2.5 ± 0.2	10.2 ± 2.6	34.3	20.9	
Treated wastewater	3.7 ± 1.3	1.5 ± 0.3	1.1 ± 0.4	0.5 ± 0.1	1.6 ± 0.5			
Zurich, Switzerland								
University hospital[e]	21.2 ± 3.6	5.6 ± 1.8	12.7 ± 2.1	3.3 ± 1.1	16.0 ± 3.3	23.1[f]	11.0[f]	Alder et al. (2004)
Kalmar, Sweden								
County hospital[g]	3.6–101							Lindberg et al. (2004)
Germany								
Five different hospitals	0.7–124.5							Hartmann et al. (1999)
University hospital, Würzburg[h]	2–51	44						Ohlsen et al. (2003)

[a] Average concentration × average wastewater volume.
[b] Load/ratio of unchanged excretion in urine/ number of patients. An excretion in urine of 55% for ciproflocacin and of 30% for norfloxacin was assumed.
[c] One grab samples of untreated water (duplicated analysis).
[d] Hourly sampling, two sampling campaigns in September 2004 and in April 2005.
[e] 24 h time proportional samples October 2002, hospital with 1000 beds, estimated wastewater discharged of 600 m^3/d.
[f] $mg\,d^{-1}\,bed^{-1}$.
[g] Grab samples of untreated water.
[h] Hourly sampling.

comparable to those measured in studies in European hospitals such as in Switzerland (0.3–29 μg l^{-1} for CIP, 0.3–8 μg l^{-1} for NOR) (Alder et al., 2004), in Sweden (3.6–101 μg l^{-1} for CIP) (Lindberg et al., 2004) and in Germany (0.7–125 μg l^{-1} for CIP, and 44 μg l^{-1} for NOR (Hartmann et al., 1999; Ohlsen et al., 2003). In a study performed in the USA, CIP and OFL occurred in hospital effluents at concentrations up to 2 μg l^{-1} and 35 μg l^{-1}, respectively (Brown et al., 2006). Among the six hospitals investigated in Hanoi, FQs showed the lowest concentrations in effluents of the hospital for cancer treatment (III), even though the number of patients was rather high and the total wastewater volume was similar to those of the other hospitals. The average CIP concentration measured in Huu Nghi hospital (VI) raw wastewater was 25.8 ± 8.1 μg l^{-1}. It was at least three times higher than those found in other hospitals. The higher CIP concentrations can be explained by a different consumption pattern in this hospital. For NOR the average concentration in raw wastewater was 8.4 ± 2.5 μg l^{-1} and thus in the same range as in the other hospital effluents.

The CIP/NOR concentration ratios in the hospitals depend on the specialization of a particular hospital, the number of patients and also on the prescription practice of the doctors. However, it is difficult to quantify the consumption of antibacterials in the hospitals because they can be provided by the hospital or can be bought in pharmacies outside the hospital and consumed by the patients in the hospital. Under this constraint, a comparison of mass fluxes and the number of patients in the different hospitals can still be attempted. Based on the measured concentrations in untreated wastewater, the number of patients, and assuming an excretion in urine of 55% for CIP and 30% for NOR, the consumption of CIP and NOR in the hospital was estimated to range from 0.5 to 34.3 mg and up to 30.7 mg per patient and per day. The highest consumption occurred in hospitals with surgical and polyclinic departments, where the estimated consumption varied from 7.6 to 34.3 mg per day and patient for CIP and from 7.5 to 30.7 mg per day and patient for NOR. Using the same approach, but considering beds instead of patients, similar values (23.1 mg CIP and 11.0 mg per bed and per day) were estimated for antibiotics consumption in the University Hospital of Zurich, Switzerland, with 1000 beds (Alder et al., 2004).

In Hanoi, FQ concentrations entering surface waters via hospital effluents ranged from 1.1 to 10.9 μg l^{-1} for CIP and from 1.5 to 15.2 μg l^{-1} for NOR. These concentrations are one to two orders of magnitude higher than the discharged concentrations in treated municipal wastewater in Switzerland (0.072 ± 0.014 μg l^{-1} CIP and 0.057 ± 0.012 μg l^{-1} NOR) (Golet et al., 2002), in Sweden (0.017 ± 0.012 μg l^{-1} CIP and 0.020 ± 0.017 μg l^{-1} NOR) (Lindberg et al., 2005) and in the USA (0.32 ± 0.10 μg l^{-1} CIP) (Batt et al., 2007). The mass flows entering surface waters via hospital effluents ranged from 0.3 to 7.7 g d^{-1} for CIP and from 0.5 to 4.6 g d^{-1} for NOR in Hanoi. The FQ loads were up to 6 times higher than the discharge of treated municipal wastewater in Switzerland (0.8 ± 0.4 g d^{-1} CIP and 0.6 ± 0.3 g d^{-1} NOR) (Golet et al., 2002) and in Sweden (1.4 ± 0.3 g d^{-1} CIP and 1.7 ± 0.4 g d^{-1} NOR) (Lindberg et al., 2006). These results confirm that untreated hospital wastewater is a significant source of antibacterials directly released into the rivers flowing through Hanoi.

3.2. Concentrations and mass flows in a hospital wastewater treatment facility

Fig. 1 shows the daily variation of CIP and NOR concentrations in wastewater samples collected in April 2005 at four sampling points along the treatment facility over a 24 h sampling period, and the levels of the 24-h composite samples. The FQs concentrations in filtered raw sewage varied from 16.0 to 44.1 μg l^{-1} for CIP and from 5.3 to 9.5 μg l^{-1} for NOR, and in filtered treated effluents from 2.5 to 5.5 μg l^{-1} for CIP and from 1.0 to 1.7 μg l^{-1} for NOR. These effluent concentrations are significantly lower in comparison to those in the untreated effluents from the other hospitals, in particular if consumption per patient is considered.

For filtered raw sewage, the concentrations fluctuated considerably (up to a factor of 3) and the highest concentration of CIP was measured at 11 p.m. This fluctuation is comparable with those reported by Ohlsen et al. (2003) (see figure in Alder et al., 2006) where the maximum concentration of CIP in raw hospital wastewater was measured at 12 p.m. The highest level of NOR in raw wastewater was measured at 5 p.m. and the daily fluctuation was less than that of CIP. The excretion of CIP and NOR depends on several factors such as time of the consumption and excretion in the urine (4 h for CIP and 6 h for NOR).

The average concentrations of CIP and NOR in hourly grab samples and also composite samples were reduced after the subsequent treatment steps (from sampling sites No. 1 to No. 4) as illustrated in Fig. 1. FQ mass flows entering the treatment plant as a dissolved fraction in filtered raw sewage were 8.9 ± 2.3 g d^{-1} for CIP and 2.0 ± 0.3 g d^{-1} for NOR. For filtered treated effluent the mass flows for CIP and NOR were 1.3 ± 0.2 g d^{-1} and 0.4 ± 0.1 g d^{-1}, respectively (Fig. 2). Thus, wastewater treatment

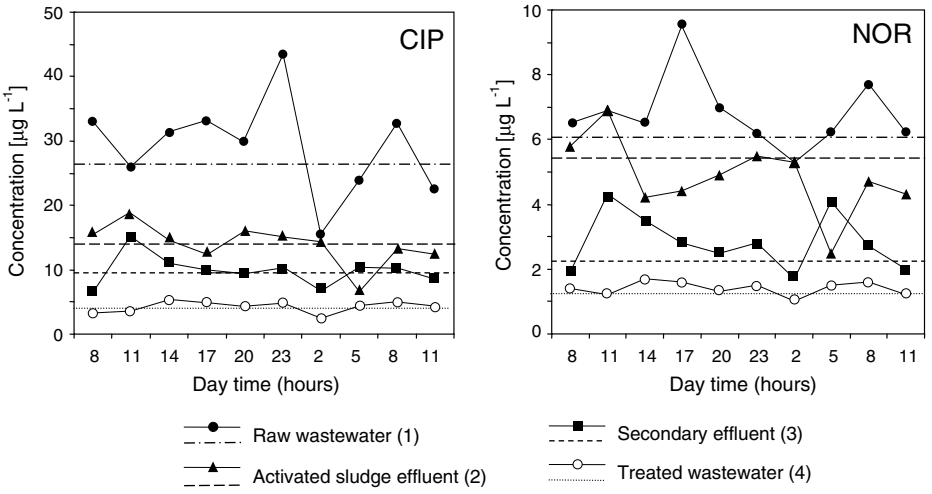

Fig. 1. Daily fluctuations of ciprofloxacin and norfloxacin concentrations in filtered wastewater collected in April 2005 at the different stages of the treatment facility of Huu Nghi hospital. The dashed lines correspond to the concentration of the 24 h composite sample.

resulted in a substantial reduction in the FQ concentrations in the aqueous phase.

Fig. 2 shows the removal in % (relative to single FQ-input) in the WWTP, which indicates the importance of each stage in the WWTP. The main removal occurs during mechanical and biological treatment (primary clarifier, activated sludge reactor and secondary clarifier), where CIP is removed up to 65 ± 9% and NOR up to 55 ± 19% from the aqueous phase. The FQs associated with suspended solids are transported via excess sludge to the sludge collection tank. The dissolved fraction in the secondary effluent flows into the anaerobic treatment where 19 ± 14% of CIP and 28 ± 3% of NOR are removed through sorption to sludge.

Overall, removal values determined for the whole process were 86% for CIP and 82% for NOR. About one third of FQs are excreted in feces (Kuhlmann et al., 1998) and therefore this percentage of FQs may also be associated with suspended solids of raw sewage. Therefore, a higher mass flow in raw sewage can be estimated, 10.3 g d^{-1} of CIP and 3.3 g d^{-1} of NOR. These values are comparable with other studies (92% for CIP and 88% for NOR) reported for the wastewater treatment plants in Switzerland (Golet et al., 2003), with results in Sweden (94% for CIP and 97% for NOR) (Lindberg et al., 2006) and in USA (59–76% for CIP) (Batt et al., 2007).

3.3. Minimum inhibitory concentration (MIC) of CIP & NOR for E. coli isolated from wastewater of the Huu Nghi hospital

The number of *E. coli* colonies existing in wastewater samples was reduced by two orders of magnitude through the treatment process. Fifteen samples, including grab and 24-h composite samples collected at Huu Nghi wastewater treatment facility in September 2004, were chosen for *E*-tests. Bacterial isolates were considered resistant to CIP or NOR if the *E*-test MIC was higher than 32 μg ml^{-1} or 256 μg ml^{-1}, respectively. Out of 15 isolates, 8 (53%) were resistant against both CIP and NOR. It was found that *E. coli* strains were resistant in all raw wastewater samples and susceptible in treated wastewater samples (Table 3). These results contrast to published data by (Reinthaler et al., 2003) reported in a study based on a total of 767 samples. In that study NOR and CIP were shown to be 100% effective towards *E. coli* in the influent and sludge of two WWTPs.

3.4. Risk characterization of hospital wastewater

In an earlier study, using acute ecotoxicity data from the literature, a risk characterization of FQs for relevant bacterial population in WWTPs (*Pseudomonas putida*) and in surface waters with regard to algae was conducted (Golet et al., 2002). Predicted no-effect concentrations $PNEC_{WWTP}$ of 8 μg l^{-1} and a $PNEC_{surface\ water}$ of 3 μg l^{-1} were calculated using EC_{50} (growth inhibition) data of CIP. The total FQs concentrations in some hospital wastewaters exceeded the calculated $PNEC_{WWTP}$ range ($MEC/PNEC_{WWTP} > 1$). Usually a dilution factor of 10 for WWTP effluents are considered for estimating concentrations in surface waters, but because in Hanoi the relative amount of wastewater discharged into the rivers is much higher, a $MEC/PNEC_{surface\ water}$ of ≈1 may be reached. However, such a risk characterization is limited to one compound. For a more advanced risk characterization, data on chronic ecotoxicity and mixture toxicities would be needed.

Table 3
Numbers of *Escherichia coli* in wastewaters Huu Nghi hospital and *E*-test results

Wastewater sample		Number of (colonies per 100 ml)	MIC (μg ml^{-1})		FQ concentration in filtered samples (μg l^{-1})	
			CIP	NOR	CIP	NOR
Raw wastewater	Grab sample 1	>100 000	>32	>256	23.2	8.9
	Grab sample 2	>100 000	>32	>256	20.5	7.8
	Grab sample 3	26 000	>32	>256	17.8	8.2
	Composite sample	>100 000	>32	>256	18.2	8.1
Activated sludge effluent	Grab sample 1	53 000	1	8	7.4	5.1
	Grab sample 2	51 000	0.25	1	7.2	5.6
	Grab sample 3	69000	>32	>256	9.2	6.9
	Composite sample	31 000	>32	>256	6.4	4.7
Secondary effluent	Grab sample 1	9000	0.064	0.19	7.7	5.3
	Grab sample 2	41 000	1	2	10.6	6.9
	Grab sample 3	22 000	>32	>256	9.7	6.4
	Composite sample	35 000	>32	>256	5.7	4.1
Treated wastewater	Grab sample 1	2000	0.75	4	2.1	2.1
	Grab sample 2	6000	0.36	1.5	1.8	1.6
	Composite sample	13 000	0.032	0.19	1.9	1.6

4. Conclusion

This study provided an overview on the occurrence of fluoroquinolone antibacterials in hospital effluents in Hanoi. The use patterns as well as the concentrations and loads in hospital effluents of fluoroquinolone antibacterials in Vietnam were comparable to those in Europe and in the USA, reflecting the ubiquitous occurrence of these pharmaceuticals. As expected, hospitals are important point sources contributing to the release of both

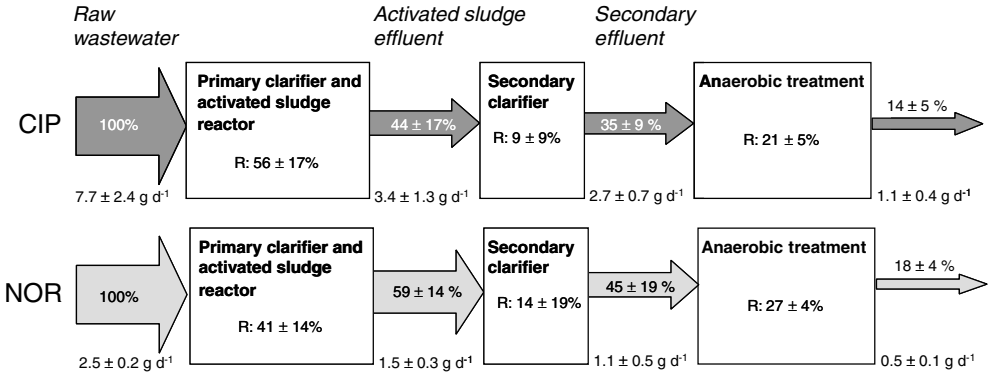

Fig. 2. Average of two days relative removals (R in %) of ciprofloxacin and norfloxacin through the wastewater treatment facility in Huu Nghi hospital (100% equal to single FQ input in the aqueous phase entering the WWTP).

antimicrobials and antibiotic resistance genes into surface waters, especially if hospital wastewaters are discharged without treatment into the receiving ambient waters. Wastewater treatment resulted in a substantial reduction of FQs entering the aquatic environment. Therefore, due to the lack of municipal WWTPs, the treating of hospital wastewater before discharging into municipal sewers should be considered a viable option and consequently be implemented.

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