Antibiotic Contamination in Wastewater Treatment Plants of a Highly Urbanized City in Vietnam: Occurrence, Removal, and Environmental Risks

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***Abstract*—This study evaluated the occurrence, removal efficiency, and environmental risks of ten commonly used antibiotics in two Municipal Wastewater Treatment Plants (WWTPs) in Ho Chi Minh City, Vietnam, one of the most densely populated urban areas in Southeast Asia. Fluoroquinolones were the most frequently detected antibiotics in influent samples, with concentrations reaching the µg/L range, followed by sulfonamides and trimethoprim. In contrast, macrolides were detected at low or undetectable levels in raw wastewater. Although overall antibiotic concentrations were significantly reduced after treatment, residual fluoroquinolones persisted in the effluent, and macrolide concentrations unexpectedly increased. Ecological risk assessment of receiving waters indicated moderate risks associated with azithromycin, levofloxacin, and ciprofloxacin. These findings underscore the limitations of conventional treatment technologies in fully mitigating antibiotic pollution and raise concerns about the potential for the propagation of antimicrobial resistance and long-term ecological harm. Further research is recommended to assess antibiotic residues in dewatered sludge, which may serve as a persistent environmental reservoir, particularly where sludge is reused or disposed of on land.**

***Keywords*—antibiotics, fluoroquinolones, macrolides, wastewater treatment plants, risk assessment**

I. Introduction

According to the WHO Report on Surveillance of Antibiotic Consumption (2016–2018), antibiotic consumption in 65 countries exceeds 12,000 metric tons annually [1]. A recent study by the One Health Trust estimated that worldwide antibiotic consumption increased by more than 21% between 2016 and 2023, with the most significant growth observed in middle-income countries [2]. Vietnam is among the countries with the highest antibiotic consumption, with an estimated 3,838 tons used in 2015. Of this total, 2,751 tons (71.7%) were allocated to animal use and 1,086 tons (28.3%) to human use [3].

Once administered, antibiotics are often incompletely metabolized, with up to 70% excreted unchanged [4]. They are frequently detected in wastewater, sometimes at concentrations in the µg/L range, and their removal depends on compound-specific properties and treatment technologies [5]. However, conventional WWTPs are not specifically designed to remove antibiotics, leading to their persistence in treated effluent. For instance, a study of 10 full-scale WWTPs across 4 cities in China found that conventional treatment processes are largely ineffective at removing antibiotics [6]. Similarly, an investigation of 48 WWTPs across 11 European countries detected 47 different antibiotics in effluent wastewater [7]. Other studies have further highlighted the presence of antibiotic residues in final effluents and their potential ecological impacts [8]. The persistence of antibiotic residues in wastewater is of major concern because it can facilitate the spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), posing risks to both environmental and public health [4, 9, 10].

In Vietnam, as in many other developing countries, concerns regarding the environmental management of antibiotics are increasing alongside their growing use in both human and veterinary medicine. Despite the country's high antibiotic consumption, data on antibiotic levels in municipal wastewater remain limited [11]. Previous research has primarily focused on antibiotic residues in hospital wastewater [12–14] or surface waters [15–17]. However, domestic wastewater treatment facilities, which are continuous sources of antibiotic discharge into the environment, have not been adequately investigated.

This study aims to address these knowledge gaps by conducting a comprehensive assessment of antibiotic residues in WWTPs in Ho Chi Minh City, Vietnam's second-largest urban center. Analyses of influent samples provide insights into community-level antibiotic consumption, while effluent analyses reflect the efficiency of treatment processes in removing these contaminants. Understanding residual antibiotic concentrations in WWTPs—a major point source of antibiotics—is essential for developing improved treatment strategies to mitigate environmental risks. Findings from this study may also contribute valuable data for wastewater-based epidemiology (WBE) approaches if relevant parameters are integrated. By filling these critical gaps, the research provides policymakers with a scientific basis for enhancing wastewater management and reducing antibiotic pollution in rapidly urbanizing regions.

II. Materials And Methods

A. Target Antibiotics

Ten antibiotics from different therapeutic classes were selected for analysis. The selection was based on the list of priority antibiotics identified in the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance, which updates the list of critically important antimicrobials for human medicine every two years. These compounds are frequently detected in wastewater environments and pose a

high risk of contributing to antimicrobial resistance.

- **Co-trimoxazole:** Sulfamethoxazole (SMX) and Trimethoprim (TMP)
- **Macrolides**: Erythromycin (ERY), Azithromycin (AZI), and Clarithromycin (CLAR)
- **Tetracyclines**: Tetracycline (TC), Oxytetracycline (OTC), and Chlortetracycline (CTC)
- **Fluoroquinolones**: Ciprofloxacin (CIPX) and Levofloxacin (LVFX)

The physicochemical properties of the target antibiotics are summarized in Table A1 (Supplementary Information). All antibiotics and analytical-grade reagents or solvents were of (> 99% purity) and purchased from Sigma-Aldrich (Singapore). The internal isotope standard sulfamethoxazole-d4 (SMX-d4) was obtained from Toronto Research Chemicals (Canada).

B. Sampling Sites and Collection Procedure

The study was conducted in Ho Chi Minh City, the second-largest city in Vietnam. Effluent wastewater samples were collected daily for 7 consecutive days in April–May 2024 from two undisclosed WWTPs (duplicated samples each day).

WWTP A employs a combination of aeration and maturation ponds to treat wastewater discharged into the polluted Nuoc Den Canal. The plant has a design capacity of 46,000 m³/day.

WWTP B—the largest facility in the city—has a design capacity of 469,000 m³/day and serves approximately two million urban residents. It employs conventional treatment processes, including activated sludge reactors and chlorination for disinfection. Effluents from this plant are discharged into the Tac Ben Ro Canal, a tributary of the Nha Be River.

Details of treatment technologies and sampling locations are presented in Figs. A1 and A2 (Supplementary Information). To inhibit microbial activity, all samples were immediately adjusted to pH 3 using 4 M H_2SO_4 upon collection. In the laboratory, samples were filtered through glass microfiber filters (Whatman GF/C, 1.2 μm), stored at 4 °C, and pretreated within 24 h.

C. Sample Pretreatment and Instrument Analysis

Sample extraction and instrumental analysis followed the procedure described by Rodriguez *et al*. [NO_PRINTED_FORM] [8] with minor modifications. Samples were extracted using solid-phase extraction (SPE) with Chromabond HR-X® cartridges (500 mg, 6 mL, Macherey-Nagel). The cartridges were preconditioned with 6 mL methanol followed by 6 mL of ultrapure water containing 0.2% formic acid at a flow rate of 6 mL/min. Filtered wastewater samples (200 mL) were then loaded at the same flow rate. The analytes were eluted with 5 mL of acetone and 2 mL methanol at 1 mL/min.

Eluates were evaporated to dryness under a gentle nitrogen stream and reconstituted in 1 mL of a methanol–ultrapure water mixture (70:30, v/v). The reconstituted extracts were filtered through 0.22 μm nylon membrane filters, transferred into autosampler vials, and stored at −20°C until UPLC–MS/MS analysis.

Chromatographic separation was achieved on a reversed-phase Kinetex Biphenyl column (2.6 μm, 100 Å, 50 2.1 mm; Phenomenex). The mobile phases consisted of ultrapure water with 0.1% formic acid (A) and acetonitrile with 0.2% formic acid (B), with detailed solvent gradient provided in Table A2 Supplementary Information). The flow rate was maintained at 0.15 mL/min, the column temperature at 40 °C, the injection volume at 10 μL, and the total run time was 11 min. Analyses were carried out using a Sciex 3500 Triple Quadrupole LC–MS/MS system (Ontario, Canada), equipped with an ExionLC AC series UPLC system.

Data acquisition and processing were performed using Analyst 1.6.1. Optimized instrumental parameters and method validation details are presented in Table A3 and Table A4 of the Supplementary Information.

D. Data Analysis and Environmental Risk Assessment

The removal efficiency was evaluated using Eq. (1):

$$\text{Removal Efficiency}=100-\left(\frac{C_{eff}}{C_{inf}}\times 100\right) \quad (1)$$

where C_{eff} represents the antibiotic concentration in the effluent, and C_{inf} is the concentration in the influent.

To assess environmental risks, this study used the same approach as previous studies. Risk Quotients (RQs) were calculated using Eq. (2):

$$\text{RQ} = (\text{PEC})/(\text{PNEC}) \quad (2)$$

where PEC is the predicted environmental concentration of the target compound, obtained by dividing the antibiotic concentrations in the effluent by 10, as calculated in the Supplementary Information—reflecting the estimated wastewater dilution factor for the two studied WWTPs in Vietnam. PNEC is the predicted no-effect concentration, sourced from the literature. Two types of PNEC values were considered.

- Environmental PNEC (PNEC-ENV) is calculated based on environmental toxicity data collected by Tell *et al.* [18].
- Minimum Inhibitory Concentration PNEC (PNEC-MIC) is estimated using data to determine the threshold antibiotic concentration in the environment below which bacterial resistance selection is unlikely to occur. The PNEC-MIC values were developed by Bengtsson-Palme and Larsson in 2016 [19].

For risk assessment, the lower of the two PNEC values was used to evaluate antibiotic contamination in the receiving waters of the two WWTPs (Table A5, Supplementary Information), ensuring both ecological safety and resistance prevention. Calculated risk levels were categorized as follows: Low risk: RQ ≤ 0.1; Moderate risk: 0.1 < RQ ≤ 1.0; High risk: RQ > 1.0.

III. Results and Discussion

A. Antibiotic Residues in Wastewater Samples

Among the ten target antibiotics, fluoroquinolones exhibited the highest concentrations in the influent wastewater of both WWTPs (Fig. 1). Sulfamethoxazole and trimethoprim ranked second, while macrolides and

tetracyclines accounted for only a small fraction. These findings are consistent with the recent review by de Ilurdoz *et al.* [20], who reported that Sulfamethoxazole (SMX) is the most frequently detected antibiotic in aquatic environments, and that ciprofloxacin, sulfamethoxazole, and trimethoprim typically occur at the highest concentrations. The dominance of specific antibiotics in influent wastewater can be attributed to several factors, including their high consumption rates, the urinary excretion fractions of the compounds in humans (Table A6), and their degradation within the sewer system.

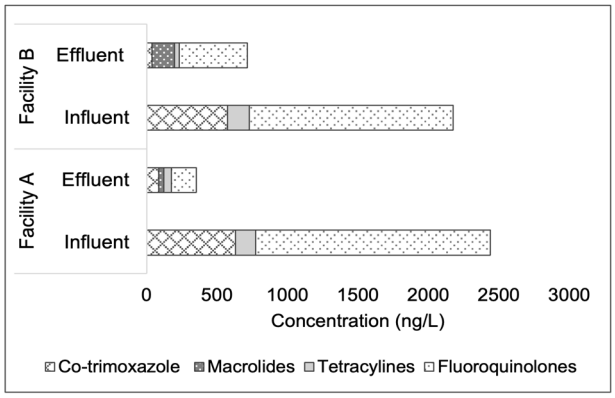

Fig. 1. Average concentration of target antibiotic in wastewater samples.

1) Co-trimoxazole (sulfamethoxazole and trimethoprim)

The average SMX concentration was similar in the two WWTPs, approximately 500 ng/L. In contrast, trimethoprim (TMP) concentrations were 134.3 ± 18.5 ng/L in WWTP A and lower in WWTP B at 54.3 ± 16.2 ng/L (Table 1). Consistent with our results, SMX and TMP have been ubiquitously detected in previous studies of wastewater-impacted urban canals in Hanoi [21, 22].

SMX and TMP are commonly co-administered as co-trimoxazole, a fixed 1:5 combination used to treat protozoan and bacterial infections [23, 24]. Thiebaut [25] proposed that the SMX/TMP concentration ratio can serve as a marker to identify the source of untreated wastewater. Ratios between 1.1 and 3.3 indicate that human consumption and excretion are the dominant sources, whereas higher ratios in livestock effluents reflect exclusive use of SMX. In this study, the SMX/TMP ratios were 3.7 in WWTP A and 9.6 in WWTP B. These elevated ratios suggest that both WWTPs likely received inputs beyond domestic sewage, such as effluents from small-scale livestock farming or slaughterhouse operations within their catchment areas—particularly for WWTP B.

2) Macrolides

Despite the high consumption of macrolides in Vietnam [26], the concentrations of three macrolide antibiotics—Azithromycin (AZI), Clarithromycin (CLAR), and Erythromycin (ERY)—were either undetected or present at low levels in the influent of both WWTPs. Approximately 90% of Vietnamese households use septic tanks as primary treatment systems, which retain raw wastewater for 48–72 h before discharging it into the drainage network. During this retention period, certain antibiotics may partially degrade before reaching the WWTPs [27]. Because macrolides are mainly excreted in feces (Table A5, Supplementary Information), they are likely to adsorb onto sediments that settle in septic tanks. Previous studies also reported that macrolides degrade more rapidly in sewer systems, primarily through hydrolysis, compared with quinolone and sulfonamide antibiotics [28].

3) Fluoroquinolones

In this study, Levofloxacin (LVFX) and Ciprofloxacin (CIPX) were detected at concentrations in the µg/L range (Table 1). Ciprofloxacin has also been reported at very high concentrations in wastewater from healthcare facilities prior to treatment in Hanoi [12, 13] and Ho Chi Minh City [14], confirming its widespread use in Vietnam. The predominance of fluoroquinolones in this study can be attributed to their high consumption rates and substantial urinary excretion fractions, which together result in their greater contribution among the target antibiotics.

4) Tetracycline Group

Compared to other antibiotic groups such as macrolides and fluoroquinolones, the use of tetracyclines for human medical purposes in Vietnam is relatively low [26]. In this study, Tetracycline (TC) was detected at notable concentrations in both WWTPs, measuring 117.2 ± 38.0 ng/L in WWTP A and 128.5 ± 86.1 ng/L in WWTP B. In contrast, Oxytetracycline (OTC) and Chlortetracycline (CTC) were frequently detected but only at low concentrations (Table 1). Similarly, Tran *et al.* [22] reported undetectable to low concentrations of tetracyclines in urban drainage water in Hanoi.

B. Removal Efficiency

1) Co-trimoxazole (Sulfamethoxazole and Trimethoprim)

Sulfamethoxazole (SMX) was effectively removed in both WWTPs (Table 1), consistent with previous studies that reported high SMX removal rates in conventional activated sludge systems [29, 30]. In contrast, trimethoprim (TMP) exhibited moderate removal, with approximately 50% removal efficiency in both WWTPs.

2) Macrolides

Interestingly, Clarithromycin (CLAR) and Azithromycin (AZI) were frequently detected in the effluents of both WWTPs (detection frequency, DF = 100%). The concentrations of CLAR and AZI in WWTP A effluent were 46.8 ± 28.4 ng/L, while those in WWTP B were approximately twice as high, at 113.6 ± 27.3 ng/L. Macrolides remain positively charged in wastewater because of their pKa values (8.7–9.5), which promotes strong electrostatic binding to the negatively charged surfaces of activated sludge particles. Their relatively high hydrophobicity (log Kow ~3.0–4.0) further enhances adsorption onto organic matter in sludge. WWTP A employs aerated lagoons and sedimentation ponds without activated sludge recirculation, whereas WWTP B operates with activated sludge recirculation. This operational difference likely contributes to the higher effluent concentrations observed WWTP B, as macrolides can occur during sludge recirculation.

3) Fluoroquinolones

The removal of fluoroquinolones was generally high. Levofloxacin (LVFX) removal exceeded 90% in WWTP A but was 50% in WWTP B. Ciprofloxacin (CIPX) removal

remained high in both WWTPs, at 86% in WWTP A and 82% in WWTP B. Fluoroquinolones are resistant to hydrolysis, heat, and biodegradation [31]. Instead, photodegradation is considered a major transformation pathway [32, 33]. Because both WWTPs in this study are in regions with abundant sunlight and year-round strong Ultraviolet (UV) radiation, photodegradation is likely an important factor in the breakdown of LVFX and CIPX.

Table 1. Occurrence, concentration, and removal efficiency of antibiotics in influent and effluent samples from WWTP A and WWTP B

	WWTP A (n=14) (ng/L)							WWTP B (n=14) (ng/L)						
	Influent			Effluent			RE	Influent			Effluent			RE
Compounds	Mean	Range	DF	Mean	Range	DF		Mean	Range	DF	Mean	Range	DF	
Sulfamethoxazole	496.6	298.3–787.6	100	30.2	2.8–57.1	100	93	518.9	155.9–721.2	100	10.5	nd–33.4	71	99
Trimethoprim	134.3	118.1–179.9	100	55.7	13.4–123.2	100	51	54.3	33.9–84.6	100	27.2	17.3–50.9	100	47
Clarithromycin	nd	nd–11.3	23	10.0	nd–17.6	69	<0	nd	nd–0	0	46.8	19.6–110.7	100	<0
Erythromycin	nd	nd–6.2	8	nd	nd–5.7	8	-	nd	nd–9.4	14	nd	nd–5.9	7	-
Azithromycin	nd	nd–16.3	38	25.2	6.9–53.9	100	<0	nd	nd–164.9	17	113.6	72.4–156.1	100	<0
Tetracycline	117.2	50.8–168.7	92	19.8	nd–36.3	77	88	128.5	13.3–267.0	100	14.9	9.7–24.4	100	76
Chlortetracycline	9.6	nd–16.1	46	14.8	nd–25.5	62	<0	10.3	8.4–13.4	100	8.3	6.3–9.4	100	18
Oxytetracycline	17.1	5.2–1188.5	100	19.3	5.1–60.3	100	<0	16.2	10.4–21.1	100	10.7	8.5–15.3	100	31
Levofloxacin	803.8	236.1–1188.5	100	54.1	nd–148.4	92	93	812.2	224.2–1488.4	100	370.0	153.7–745.0	100	49
Ciprofloxacin	859.8	420.8–1690.3	100	123.3	17.9–276.9	100	86	635.1	93.7–968.3	100	112.6	25.4–234.4	100	82

Note: -: no difference between influent and effluent; nd: not detected; RE: removal efficiency

Additionally, fluoroquinolones exhibit strong adsorption to sludge despite their relatively low log K_{ow} values. Jia *et al.* [31] reported that 50–87% of the initial fluoroquinolone load in a Chinese WWTP was ultimately retained in dewatered sludge. Partition coefficients of approximately log K_d ~4 have been reported elsewhere, suggesting that sludge can act as a significant reservoir for fluoroquinolone antibiotics [34, 35].

4) Tetracycline Group

Tetracycline (TC) showed high removal efficiencies in both WWTPs - 88% in WWTP A and 76% in WWTP B. These results align with previous studies in activated sludge systems, which also reported high TC removal efficiencies [30, 36]. Photodegradation may further enhance TC removal, as wastewater containing photosensitizers such as Dissolved Organic Matter (DOM), Nitrate (NO_3^-), and Nitrite (NO_2^-) promotes light-induced degradation [37]. Despite this apparent removal from the aqueous phase, TC is known to strongly adsorb to sludge, forming stable complexes with Ca^{2+} and other metal ions, with up to 90% of the compound bound to solid phases [37].

C. Environmental Risk Assessment

Calculated Risk Quotient (RQ) values are presented in Table 2. The RQ ranged from 0.002 to 0.568, indicating that most of the 10 antibiotics analyzed in this study posed a low ecological risk (RQ < 0.1) to aquatic organisms. However, azithromycin (RQ = 0.568), ciprofloxacin (RQ = 0.412), and levofloxacin (RQ = 0.247) exhibited moderate risk levels (0.1 < RQ < 1).

Table 2. Predicted antibiotic resistance and ecotoxicity in environmental water receiving effluent from WWTP A and WWTP B

		WWTP A			WWTP B		
Antibiotic	PNEC value[a] (µg/L)	PEC (µg/L)	Risk Quotient	Risk level	PEC (µg/L)	Risk Quotient	Risk level
Sulfamethoxazole	0.6	0.003	0.005	low	0.001	0.002	low
Trimethoprim	0.5	0.006	0.011	low	0.003	0.005	low
Clarithromycin	0.08	0.001	0.013	low	0.005	0.059	low
Erythromycin	0.5	nd	na	low	nd	na	low
Azithromycin	0.02	0.003	0.126	intermediate	0.011	0.568	intermediate
Tetracycline	1	0.002	0.002	low	0.001	0.001	low
Chlortetracycline	na	0.001	na	na	0.001	na	na
Oxytetracycline	0.5	0.002	0.004	low	0.001	0.002	low
Levofloxacin	0.25	0.005	0.022	low	0.037	0.148	intermediate
Ciprofloxacin	0.06	0.012	0.205	intermediate	0.011	0.188	intermediate

[a] derived from Table A6 in the supplementary information

These antibiotics are bioactive even at low concentrations and may influence aquatic microbial communities and the development of Antibiotic Resistance Genes (ARGs) [38, 39]. Azithromycin, a macrolide, tends to persist longer in aquatic environments because of its chemical stability, which aligns with its relatively higher RQ [40, 41]. Ciprofloxacin and levofloxacin (fluoroquinolones) are strongly adsorbed to sediments, so their ecological effects may extend beyond the

water column into benthic organisms and sediment microbiota [42, 43].

A previous study across seven European countries similarly identified moderate environmental risks for several antibiotics, including cephalexin, ciprofloxacin, and azithromycin, in effluent wastewater among 53 monitored compounds [8]. Furthermore, ciprofloxacin, azithromycin, erythromycin, and clarithromycin are listed in the 2018 update of the EU Surface Water Watch List under the Water Framework Directive, reflecting their potential ecological concern.

In Vietnam, the moderate risk observed for these antibiotics may be exacerbated by limited wastewater treatment efficiency and high antibiotic consumption rates, which can enhance the persistence and bioaccumulation of residues in surface waters. Long-term exposure to sub-lethal concentrations may contribute to the development of antimicrobial resistance and disrupt trophic interactions. Therefore, continuous monitoring and optimization of wastewater treatment processes are essential to mitigate antibiotic discharge and safeguard the integrity of aquatic ecosystems and public health. Further research should also address mixture toxicity and the cumulative impacts of multiple antibiotics to provide a more comprehensive risk assessment for Vietnam's aquatic environments.

IV. Limitations and Future Directions

This study has several limitations. First, sample collection was conducted using grab sampling because a composite autosampler was unavailable. To account for temporal variations, samples were collected over a one-week period to capture fluctuations. Second, this study focused exclusively on antibiotic concentrations in the aqueous phase. Future research should also quantify antibiotic mass loads in sludge, as many compounds are known to strongly adsorb onto solid particles. Finally, the number of antibiotics analyzed was limited to ten compounds. Expanding future investigation to include a broader range of antibiotic classes and the transformation products of parent compounds would provide a more comprehensive understanding of emerging contaminants discharged from WWTPs. These transformation byproducts may also pose significant environmental and human health risks.

V. Conclusion

This study investigated the occurrence of 10 priority antibiotics in the influent and effluent of two WWTPs in Ho Chi Minh City. Among the target antibiotics, fluoroquinolones were the most dominant group in the influent of both WWTPs, followed by sulfamethoxazole (SMX) and trimethoprim (TMP), whereas macrolides and tetracyclines contributed only a small fraction. The high SMX/TMP ratio observed in both WWTPs suggests additional wastewater inputs beyond domestic sewage, potentially from livestock-related or industrial sources.

Overall, both WWTPs significantly reduced antibiotic concentrations. However, a decline in concentration does not necessarily reflect complete removal, as parent compounds may undergo transformation into metabolite degradation products or remain adsorbed within dewatered sludge.

Despite the reduction, the effluents from both WWTPs still contained notable levels of fluoroquinolones and macrolides, posing moderate ecological risks. Azithromycin, ciprofloxacin, and levofloxacin had the highest quotients, with azithromycin posing the greatest concern. This issue is more pronounced in WWTP B, which has a treatment capacity approximately 10 times that of WWTP A (469,000 m^3/day versus 46,000 m^3/day). Consequently, the continuous discharge of persistent antibiotic residues from WWTP B's effluent represents a substantial environmental concern.

More critically, only 8–28% of Ho Chi Minh City's municipal wastewater is currently treated centrally. Given the widespread use of fluoroquinolones and macrolides in Vietnam, effective sludge management—both in septic tanks and centralized WWTPs—is essential to mitigate environmental impacts. Continuous monitoring, degradation studies, and assessments of antibiotic resistance are urgently needed to safeguard aquatic ecosystems and public health.

Appendix

A. Information of Facilities

At the time of the study, only three wastewater treatment plants (WWTPs) were in operation in Ho Chi Minh city, treating approximately 600,000 m^3 of wastewater per day, 20% of the city's total wastewater. The planning and development of additional WWTPs in Ho Chi Minh City are still ongoing, as outlined in Decision 24/QD-TTg, issued by the Prime Minister on January 6, 2010.

WWTP A employs a combination of aeration ponds and maturation ponds to treat wastewater discharged into the polluted Nuoc Den Canal. The design capacity of this plant is 46,000 m^3/day.

WWTP B utilizes conventional treatment processes, including activated sludge reactors and chlorination for wastewater disinfection. Effluents from this plant are discharged into the Tac Ben Ro Canal, a tributary of the Nha Be River. Serving approximately 2 million urban inhabitants, WWTP B is the largest of the operating facilities in Ho Chi Minh city, with a design capacity of 469,000 m^3/day.

B. Dilution Factor of Effluent

The dilution factor (the ratio of the receiving water flow to the wastewater discharge flow) represents the extent to which wastewater is diluted when discharged into a receiving water body. It is calculated according to QCVN 24:2009/BTNMT National Technical Regulation on Industrial Wastewater. The coefficient $K_q = 0.9$ is applied when no direct flow rate data is available, corresponding to a standard assumed flow of 50 m^3/s.

In this case:

- The receiving water flow rate is 50 m^3/s, which is equivalent to 4,320,000 m^3 per 24 h.
- The wastewater discharge flow is 469,000 m^3 per 24 h, which corresponds to the facility's design capacity.

The dilution factor is determined as:

$$\text{Dilution factor} = \frac{\text{Receiving water flow}}{\text{Waste discharge flow}} = \frac{4{,}320{,}000}{469{,}000} \sim 10$$

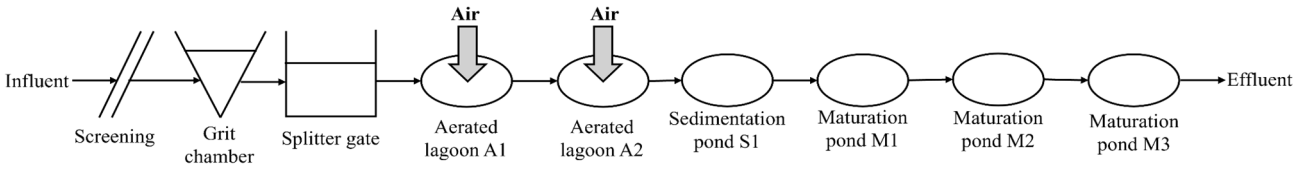

Fig. A1. Flow charts of technological processes of WWTP A.

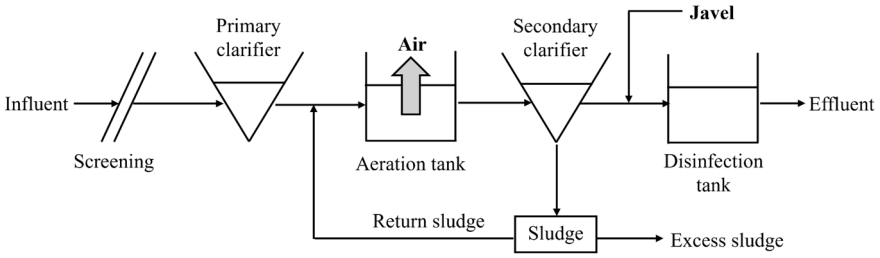

Fig. A2. Flow charts of technological processes of WWTP B.

Table A1. Physicochemical properties of the target antibiotics in this study

Class	Compound	Abbr.	Formula	MW	pK_a	$LogK_{ow}$
Sulfonamides	**Sulfamethoxazole**	SMX	$C_{10}H_{11}N_3O_3S$	253.3	1.85 5.6	0.89
Diaminopyrimidines	**Trimethoprim**	TMP	$C_{14}H_{18}N4O_3$	290.3	1.32 7.45	0.91
Macrolides	**Clarithromycin**	CLAR	$C_{38}H_{69}NO_{13}$	747.9	8.99	3.16
	Erythromycin	ERY	$C_{37}H_{67}NO_{13}$	733.9	8.88	3.06
	Azithromycin	AZI	$C_{38}H_{72}N_2O_{12}$	749.0	8.74 9.5	4.02
Tetracyclines	**Tetracycline**	TC	$C_{22}H_{24}N_2O_8$	444.4	3.3 7.8 9.6	–1.3
	Chlortetracycline	CTC	$C_{22}H_{23}CIN_2O_8$	478.9	3.3 7.6 9.3	–0.62
	Oxytetracycline	OTC	$C_{22}H_{24}N_2O_9$	460.4	3.2 7.5 8.9	– 0.9
Fluoroquinolones	**Levofloxacin**	LVFX	$C_{18}H_{20}FN_3O_4$	361.4	5.33 8.07	–0.39
	Ciprofloxacin	CIPX	$C_{17}H_{18}FN_3O_3$	331.4	5.76 8.68	0.28

MW: Molecular weight; pK_a: acidity constant; K_{ow}: the octanol-water partition coefficient

Table A2. Solvent program of liquid chromatography method

Time (min)	Flow (ml/min)	Solvent A (%)	Solvent B (%)
0.00	0.15	95	5
1.00	0.15	95	5
2.00	0.15	60	40
5.00	0.15	5	95
8.00	0.15	5	95
8.10	0.15	95	5
11.00	0.15	95	5

Mobile phase A: ultrapure water with 0.1% formic acid; Mobile phase B: Acetonitrile with 0.2% formic acid.
Flow rate: 0.15 mL min-1
Column temperature was 40 °C.

Table A3. The optimized parameters of UPLC-MS/MS

Class	Compound	Abbr.	RT (min)	Q1 mass (m/z)	Q3 mass (m/z)	ESI	DP (V)	CE (eV)	EP (V)	CXP (V)
Sulfonamides	Sulfamethoxazole	SMX	6.37	253.9	92.20	+	80	38	10	10
Macrolides	Clarithromycin	CLAR	5.82	375.2	591.3	+	100	20	10	10
	Erythromycin	ERY	6.16	734.5	576.3	+	120	29	10	10
	Azithromycin	AZI	6.46	748.3	158.1	+	120	36	10	10
Diaminopyrimidines	Trimethoprim	TMP	5.79	445.0	410.0	+	100	30	10	10
Tetracyclines	Tetracycline	TC	5.96	479.2	444.0	+	100	32	10	10
	Chlortetracycline	CTC	5.81	461.1	426.2	+	80	26	10	10
	Oxytetracycline	OTC	5.79	362.0	318.2	+	100	30	10	10
Fluoroquinolones	Levofloxacin	LVFX	5.79	331.8	314.2	+	100	30	10	10
	Ciprofloxacin	CIPX	5.80	291.2	230.1	+	115	34	10	10

Abbr.: abbreviation; Q1 is set as the mass spectrometry 1 in which a precursor ion is selected and then is subjected to dissociation in a collision cell; Q3 is as the mass spectrometry 2 in which a preferable product ion is selected and detected; RT: retention time; ESI: electrospray ionization; DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential

Table A4. Method validation parameters

	Calibration curve equation	Linearity R^2	LOD (ng/L)	H%
SMX	$y = 0.565x + 0.029$	0.997	2.0	118.8%
CLAR	$y = 0.4491x + 0.0452$	0.984	2.8	128.3%
ERY	$y = 0.4481x + 0.0052$	0,996	4.8	34.8%
AZI	$y = 1.1511x – 0.1005$	0.998	4.8	85.1%%
TMP	$y = 1.6264x + 0.1846$	0.980	4.4	119.1%
TC	$y = 0.8147x – 0.124$	0.996	4.4	101.6%
CTC	$y = 0.5872x – 0.0922$	0.992	5.6	117.4%
OTC	$y = 0.9292x – 0.1396$	0.993	3.6	91.2%
LVFX	$y = 0.9293x – 0.0898$	0.993	6.0	73.3%
CIPX	$y = 0.4233x – 0.0538$	0.992	5.2	130.0%

Calibration curves were constructed at eight concentration levels (0.5–100 ng/mL for all analytes) using a least-squares regression model, with peak area ratios (analyte/internal standard) plotted against theoretical concentrations. The internal standard concentration was fixed at 10 ng/mL for each calibration point. All solutions were extracted using syringes, prepared in vials, and diluted with a solvent mixture of MeOH: H_2O at a 1:1 ratio.

Table A5. Predicted no-effect concentration sourced from the literature [18]

Antibiotic	PNEC-ENV (µg/L)	PNEC-MIC (µg/L)	Lowest PNEC Value (µg/L)
Sulfamethoxazole	0.6	16	0.6
Trimethoprim	100	0.5	0.5
Clarithromycin	0.08	0.25	0.08
Erythromycin	0.5	1	0.5
Azithromycin	0.02	0.25	0.02
Tetracycline	3.2	1	1
Chlorotetracycline	na	na	na
Oxytetracycline	18	0.5	0.5
Levofloxacin	n/a	0.25	0.25
Ciprofloxacine	0.45	0.06	0.06

Limit of Detection (LOD): LOD is determined based on the standard deviation of seven replicates, using an HR-X extraction column and conducted on samples with low analyte concentrations. The formula for calculating the limit of detection is: LOD=3×SD.

Recovery Efficiency (H%): The experiment was conducted using water samples by spiking 100 mL of filtered water with a mixed standard at concentration of 10 ng/L. RE were calculated using following equation:

$$H\% = \frac{C_{\text{spiked sample}} - C_{\text{sample}}}{C_{\text{standard}}} \times 100$$

$C_{\text{spiked sample}}$: concentration of analyte in spiked sample (ng/mL)

C_{sample}: concentration of analyte in sample (ng/mL)

C_{standard}: concentration of added standard in sample (ng/mL)

Table A6. AwaRe Classification and Excretion rates and pathway of target antibiotic in this study

Compound	Abbr	AwaRe Classification	Excretion rates and pathway	References
Sulfamethoxazole	SMX	Access	15–30% excreted unchanged in urine 50–70% as acetylated metabolite	[44, 45]
Trimethoprim	TMP	Access	50–60% excreted unchanged in urine	[44, 45]
Erythromycin	ERY	Watch	Mainly via bile 5–10% excreted unchanged in urine	[46]
Azithromycin	AZI	Watch	Excreted largely unchanged in feces, 6–12 % eliminated via urine.	[47]
Clarithromycin	CLAR	Watch	Metabolized in the liver, with both the parent drug and its metabolites excreted in urine and feces 25%	[46]
Tetracycline	TC	Access	80-90% excreted unchanged, through urine and feces	[44]
Oxytetracycline	OTC	Watch	60-70% excreted unchanged, through urine and feces	[48]
Chlortetracycline	CTC	Watch	mainly in feces	[49]
Ciprofloxacin	CIPX	Watch	>80% excreted unchanged through urine	[44]
Levofloxacin	LVFX	Watch	>80% excreted unchanged through urine	[50]

WHO developed a framework based on three different categories – Access, WAtch and Reserve – which all together forms the AWaRe categorization of antibiotics. *"Access" includes first- or second-choice antibiotics with optimal therapeutic value and low resistance risk; "Watch" antibiotics are for specific infections, with higher resistance potential, requiring stewardship; "Reserve" drugs are last-resort options for severe, multi-drug-resistant infections.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors have contributed to the design of this study and the compilation of the article. Do Thi Thuy Quyen: Conceptualization; Investigation, writing- original draft. Tran Thi Yen Nhi: Analysis methodology; data curation. To Thi Hien: Supervision; Writing - review and editing.

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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REFERENCES

[1] WHO. (2018). WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation. [Online]. Available: http://apps.who.int/iris

[2] E. Y. Klein *et al.*, "Global trends in antibiotic consumption during 2016-2023 and future projections through 2030," *Proc Natl Acad Sci U S A*, vol. 121, no. 49, 2024. doi: 10.1073/pnas.2411919121.

[3] J. J. Carrique-Mas, M. Choisy, N. Van Cuong, G. Thwaites, and S. Baker, "An estimation of total antimicrobial usage in humans and animals in Vietnam," *Antimicrob Resist Infect Control*, vol. 9, no. 1, p. 16, 2020. doi: 10.1186/s13756-019-0671-7

[4] K. Kummerer, "Antibiotics in the aquatic environment-a review-part I," *Chemosphere*, vol. 75, no. 4, pp. 417–434, 2009. doi: 10.1016/j.chemosphere.2008.11.086

[5] N. Le-Minh, S. J. Khan, J. E. Drewes, and R. M. Stuetz, "Fate of antibiotics during municipal water recycling treatment processes," *Water Res*, vol. 44, no. 15, pp. 4295–4323, 2010. doi: 10.1016/j.watres.2010.06.020

[6] X.-M. Guo *et al.*, "Comprehensive assessment of 45 antibiotics in ten urban wastewater treatment plants in Northeastern China: Terminal treatment is not a reliable guard," *J Hazard Mater*, vol. 489, 2025. doi: 10.1016/j.jhazmat.2025.137755

[7] K. Ng, N. A. Alygizakis, N. S. Thomaidis, and J. Slobodnik, "Wide-scope target and suspect screening of antibiotics in effluent wastewater from wastewater treatment plants in Europe," *Antibiotics (Basel)*, vol. 12, no. 1, 2023. doi: 10.3390/antibiotics12010100

[8] S. Rodriguez-Mozaz *et al.*, "Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment," *Environ Int*, vol. 140, 105733, 2020. doi: 10.1016/j.envint.2020.105733

[9] S. S. Sambaza and N. Naicker, "Contribution of wastewater to antimicrobial resistance: A review article," *J Glob Antimicrob Resist*, vol. 34, pp. 23–29, 2023. doi: 10.1016/j.jgar.2023.05.010

[10] A. M. Voigt *et al.*, "Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater — An evaluation of clinical influences," *Chemosphere*, vol. 241, 125032, 2020. doi: 10.1016/j.chemosphere.2019.125032

[11] V. N. Binh, N. Dang, N. T. K. Anh, L. X. Ky, and P. K. Thai, "Antibiotics in the aquatic environment of Vietnam: Sources, concentrations, risk and control strategy," *Chemosphere*, vol. 197, pp. 438–450, 2018. doi: 10.1016/j.chemosphere.2018.01.061

[12] L. T. Lien *et al.*, "Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use-A one year study from Vietnam," *Int J Environ Res Public Health*, vol. 13, no. 6, 2016. doi: 10.3390/ijerph13060588

[13] P. K. Thai *et al.*, "Occurrence of antibiotic residues and antibiotic-resistant bacteria in effluents of pharmaceutical manufacturers and other sources around Hanoi, Vietnam," *Sci Total Environ*, vol. 645, pp. 393–400, 2018. doi: 10.1016/j.scitotenv.2018.07.126

[14] T. D. Vo *et al.*, "Investigation of antibiotics in health care wastewater in Ho Chi Minh City, Vietnam," *Environ Monit Assess*, vol. 188, no. 12, p. 686, 2016. doi: 10.1007/s10661-016-5704-6

[15] N. Da Le *et al.*, "Antibiotic and antiparasitic residues in surface water of urban rivers in the Red River Delta (Hanoi, Vietnam): Concentrations, profiles, source estimation, and risk assessment," *Environ Sci Pollut Res Int*, vol. 28, no. 9, pp. 10622–10632, 2021. doi: 10.1007/s11356-020-11329-3

[16] N. D. Le *et al.*, "Occurrence and ecological risks of antibiotics and antiparasitics in surface water in urban lakes in Hanoi city, Vietnam," *Environ Sci Pollut Res Int*, 2024. doi: 10.1007/s11356-024-35726-0

[17] N. H. Tran *et al.*, "Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Hanoi, Vietnam," *Sci Total Environ*, vol. 692, pp. 157–174, 2019. doi: 10.1016/j.scitotenv.2019.07.092

[18] J. Tell *et al.*, "Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations," *Integr Environ Assess Manag*, vol. 15, no. 3, pp. 312–319, 2019. doi: 10.1002/ieam.4141

[19] J. Bengtsson-Palme and D. G. J. Larsson, "Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation," *Environ Int*, vol. 86, pp. 140–149, 2016. doi: 10.1016/j.envint.2015.10.015

[20] M. S. de Ilurdoz, J. J. Sadhwani, and J. V. Reboso, "Antibiotic removal processes from water & wastewater for the protection of the aquatic environment — a review," *J Water Process Eng*, vol. 45, Feb. 2022. doi: 10.1016/j.jwpe.2021.102474

[21] P. T. Hoa *et al.*, "Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam," *Sci Total Environ*, vol. 409, no. 15, pp. 2894–2901, 2011. doi: 10.1016/j.scitotenv.2011.04.030

[22] N. H. Tran, M. Reinhard, and K. Y.-H. Gin, "Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review," *Water Res*, vol. 133, pp. 182–207, 2018. doi: 10.1016/j.watres.2017.12.029

[23] A. J. Salter, "Trimethoprim-sulfamethoxazole in treatment of severe infections," *Rev Infect Dis*, vol. 4, no. 2, pp. 338–350, 1982. doi: 10.1093/clinids/4.2.338

[24] Trimethoprim and sulfamethoxazole. *MSD Manual Professional Version*. [Online]. Available: https://www.msdmanuals.com/home/infections/antibiotics/trimethoprim-and-sulfamethoxazole

[25] T. Thiebault, "Sulfamethoxazole/Trimethoprim ratio as a new marker in raw wastewaters: A critical review," *Sci Total Environ*, vol. 715, 136916, 2020. doi: 10.1016/j.scitotenv.2020.136916

[26] V. Q. Dat, "Antibiotic use in public hospitals in Vietnam between 2018 and 2022: a retrospective study," *BMJ Open*, vol. 14, no. 8, e087322, 2024. doi: 10.1136/bmjopen-2024-087322

[27] D. T. T. Quyen, T. T. Y. Nhi, N. T. T. Nhon, and T. T. Hien, "Estimating alcohol and tobacco consumption of university students and urban population in Ho Chi Minh City by wastewater analysis," *Water Environ Res*, vol. 96, no. 7, e11083, 2024. doi: 10.1002/wer.11083

[28] S. Zheng *et al.*, "Current progress in natural degradation and enhanced removal techniques of antibiotics in the environment: A review," *Int J Environ Res Public Health*, vol. 19, no. 17, 2022. doi: 10.3390/ijerph191710919

[29] O. L. Oharisi, S. Ncube, H. Nyoni, M. L. Madikizela, O. J. Olowoyo, and B. R. Maseko, "Occurrence and prevalence of antibiotics in wastewater treatment plants and effluent receiving rivers in South Africa using UHPLC-MS determination," *J Environ Manage*, vol. 345, 118621, 2023. doi: 10.1016/j.jenvman.2023.118621

[30] L. J. Zhou *et al.*, "Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China," *Sci Total Environ*, vol. 452–453, pp. 365–376, 2013. doi: 10.1016/j.scitotenv.2013.03.010

[31] A. Jia, Y. Wan, Y. Xiao, and J. Hu, "Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant," *Water Res*, vol. 46, no. 2, pp. 387–394, 2012. doi: 10.1016/j.watres.2011.10.055

[32] M. Sturini, A. Speltini, F. Maraschi, L. Pretali, E. N. Ferri, and A. Profumo, "Sunlight-induced degradation of fluoroquinolones in wastewater effluent: Photoproducts identification and toxicity," *Chemosphere*, vol. 134, pp. 313–318, 2015. doi: 10.1016/j.chemosphere.2015.04.081

[33] M. Sturini *et al.*, "Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight," *Appl Catal B*, vol. 119–120, pp. 32–39, 2012. doi: 10.1016/j.apcatb.2012.02.008

[34] E. M. Golet, I. Xifra, H. Siegrist, A. C. Alder, and W. Giger, "Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil," *Environ Sci Technol*, vol. 37, no. 15, pp. 3243–3249, 2003. doi: 10.1021/es0264448

[35] I. Senta, S. Terzic, and M. Ahel, "Occurrence and fate of dissolved and particulate antimicrobials in municipal wastewater treatment," *Water Res*, vol. 47, no. 2, pp. 705–714, 2013. doi: 10.1016/j.watres.2012.10.041

[36] K. G. Karthikeyan and M. T. Meyer, "Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA," *Sci Total Environ*, vol. 361, no. 1–3, pp. 196–207, 2006. doi: 10.1016/j.scitotenv.2005.06.030

[37] F. Ahmad, D. Zhu, and J. Sun, "Environmental fate of tetracycline antibiotics: degradation pathway mechanisms, challenges, and perspectives," *Environ Sci Eur*, vol. 33, no. 1, 2021. doi: 10.1186/s12302-021-00505-y

[38] N. Kraupner *et al.*, "Selective concentration for ciprofloxacin resistance in Escherichia coli grown in complex aquatic bacterial biofilms," *Environ Int*, vol. 116, pp. 255–268, Jul. 2018. doi: 10.1016/j.envint.2018.04.029

[39] F. Adachi, A. Yamamoto, K.-I. Takakura, and R. Kawahara, "Occurrence of fluoroquinolones and fluoroquinolone-resistance genes in the aquatic environment," *Science of The Total Environment*, vol. 444, pp. 508–514, Feb. 2013. doi: 10.1016/j.scitotenv.2013.01.077

[40] M. L. Vermillion Maier and R. S. Tjeerdema, "Azithromycin sorption and biodegradation in a simulated California river system," *Chemosphere*, vol. 190, pp. 471–480, Jan. 2018. doi: 10.1016/j.chemosphere.2017.10.008

[41] C. Mejías, J. Martín, L. Martín-Pozo, J. L. Santos, I. Aparicio, and E. Alonso, "Adsorption of macrolide antibiotics and a metabolite onto polyethylene terephthalate and polyethylene microplastics in aquatic environments," *Antibiotics*, vol. 13, no. 5, 408, Apr. 2024. doi: 10.3390/antibiotics13050408

[42] B. Zhang *et al.*, "Insight into different adsorption behaviors of twofluoroquinolone antibiotics by sediment aggregation fractions," *Environ Sci Pollut Res*, vol. 30, no. 9, pp. 24329–24343, Nov. 2022. doi: 10.1007/s11356-022-23947-0

[43] O. F. Becerra-Rueda, G. M. Rodríguez-Figueroa, A. J. Marmolejo-Rodríguez, S. Aguíñiga-García, and J. C. Durán-Álvarez, "Pharmaceutical residues in sediments of a coastal lagoon in Northwest Mexico—Occurrence and environmental risk assessment," *J Xenobiot*, vol. 14, no. 4, pp. 1757–1770, Nov. 2024. doi: 10.3390/jox14040093

[44] P. K. Jjemba, "Excretion and ecotoxicity of pharmaceutical and personal care products in the environment," *Ecotoxicology and Environmental Safety*, vol. 63, no. 1, pp. 113–130, Jan. 2005. doi: 10.1016/j.ecoenv.2004.11.011

[45] T. Thiebault, "Sulfamethoxazole/Trimethoprim ratio as a new marker in raw wastewaters: A critical review," *The Science of the Total Environment*, vol. 715, Jan. 2020. doi: 10.1016/j.scitotenv.2020.136916

[46] S. Mompelat, B. L. Bot, and O. Thomas, "Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water," *Environment International*, vol. 35, no. 5, pp. 803–814, Dec. 2008. doi: 10.1016/j.envint.2008.10.008

[47] E. Singlas, "Pharmacocinétique clinique de l'azithromycine (Clinical pharmacokinetics of azithromycin," *Pathologie-biologie*, vol. 43, no. 6, pp. 505–511, 1995.

[48] S Mompelat *et al.*, "Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water," *Environment International*, vol. 35, no. 5, pp. 803–814, 2009. doi: 10.1016/j.envint.2008.10.008

[49] Chlortetracycline. *DrugBank*. [Online]. Available: https://www.drugbank.ca/drugs/DB09093

[50] D. Czock *et al.*, "Pharmacokinetics of moxifloxacin and levofloxacin in intensive care unit patients who have acute renal failure and undergo extended daily dialysis," *Clinical Journal of the American Society of Nephrology*, vol. 1, no. 6, pp. 1263–1268, Sep. 2006. doi: 10.2215/cjn.01840506