

## Article

# Evaluation of the Correspondence between the Concentration of Antimicrobials Entering Sewage Treatment Plant Influent and the Predicted Concentration of Antimicrobials Using Annual Sales, Shipping, and Prescriptions Data

Takashi Azuma <sup>1,\*</sup>, Takashi Nakano <sup>2</sup>, Ryuji Koizumi <sup>3</sup>, Nobuaki Matsunaga <sup>3</sup>, Norio Ohmagari <sup>3,4</sup> and Tetsuya Hayashi <sup>1,5</sup>

- <sup>1</sup> Department of Environment and Health Sciences, Faculty of Pharmacy, Osaka Medical and Pharmaceutical University, Takatsuki 569-1094, Japan; t.hayashi@soai.ac.jp
- <sup>2</sup> Department of Microbiology and Infection Control, Faculty of Medicine, Osaka Medical and Pharmaceutical University, Takatsuki 569-8686, Japan; tnakano@ompu.ac.jp
- <sup>3</sup> AMR Clinical Reference Center, National Center for Global Health and Medicine, Tokyo 162-8655, Japan; rykoizumi@hosp.ncgm.go.jp (R.K.); nomatsunaga@hosp.ncgm.go.jp (N.M.); nohmagari@hosp.ncgm.go.jp (N.O.)
- <sup>4</sup> Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo 162-8655, Japan
- <sup>5</sup> Department of Food and Nutrition Management Studies, Faculty of Human Development, Soai University, Osaka 559-0033, Japan
- \* Correspondence: takashi.azuma@ompu.ac.jp; Tel./Fax: +81-72-690-1055



**Citation:** Azuma, T.; Nakano, T.; Koizumi, R.; Matsunaga, N.; Ohmagari, N.; Hayashi, T. Evaluation of the Correspondence between the Concentration of Antimicrobials Entering Sewage Treatment Plant Influent and the Predicted Concentration of Antimicrobials Using Annual Sales, Shipping, and Prescriptions Data. *Antibiotics* **2022**, *11*, 472. <https://doi.org/10.3390/antibiotics11040472>

Academic Editor: Manuela Oliveira

Received: 9 March 2022

Accepted: 30 March 2022

Published: 1 April 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** The accuracy and correspondence between the measured concentrations from the survey and predicted concentrations on the basis of the three types of statistical antimicrobial use in Japan was evaluated. A monitoring survey of ten representative antimicrobials: ampicillin (APL), cefdinir (CDN), cefpodoxime proxetil (CPXP), ciprofloxacin (CFX), clarithromycin (CTM), doxycycline (DCL), levofloxacin (LFX), minocycline (MCL), tetracycline (TCL), and vancomycin (VMC), in the influent of sewage treatment plant (STP) located in urban areas of Japan, was conducted. Then, the measured values were verified in comparison with the predicted values estimated from the shipping volumes, sales volumes, and prescription volumes based on the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB). The results indicate that the correspondence ratios between the predicted concentrations calculated on the basis of shipping and NDB volumes and the measured concentrations (predicted concentration/measured concentration) generally agreed for the detected concentration of antimicrobials in the STP influent. The correspondence ratio on the basis of shipping volume was, for CFX, 0.1; CTM, 2.9; LFX, 0.5; MCL, 1.9; and VMC, 1.7, and on the basis of NDB volume the measured concentration was CFX, 0.1; CTM, 3.7; DCL, 0.4; LFX, 0.7; MCL, 1.9; TCL, 0.6; and VMC, 1.6. To our knowledge, this is the first report to evaluate the accuracy of predicted concentrations based on sales, shipping, NDB statistics and measured concentrations for antimicrobials in the STP influent.

**Keywords:** antimicrobials; sewage influent; wastewater treatment plant (WWTP); predicted environmental concentration; national database of health insurance claims and specific health checkups of Japan (NDB)

## 1. Introduction

The emergence and spread of antimicrobial-resistant bacteria (AMRB) is rapidly becoming an ongoing problem on a global scale [1–3]. The problem of AMRB has been assessed and addressed in medical facilities such as hospitals and livestock [4–6]. However, AMRB are widely detected in aquatic environments such as rivers, lakes, and seas [7–9],

and sewage treatment plants (STPs), where various wastewaters are concentrated in sewage and have been noted as a potentially important source of AMRB [10–12].

Previous studies have detected antimicrobials, as well as AMRB, in wastewater [13,14]. These antimicrobials are mainly excreted from the body in the form of unchanged pairs that retain their original medicinal effects, and they are collected in STPs via the sewage system [15,16]. On the other hand, conventional wastewater treatment in STPs cannot completely remove chemical pollutants such as pharmaceuticals, and the treated water flows into the aquatic environments, such as rivers, lakes, and oceans [13,17]. The effects of these antimicrobial residues in these aquatic environments include not only toxic effects (growth inhibition, suppression of reproduction, and genotoxicity, etc.) on ecosystems but also on human health (allergy induction, dysbiosis on the gut flora, decreased immune system, and unexpected loss of healthy life expectancy, etc.) through drinking water sources, and these residues also potentially promote the development of new AMRB, thereby increasing the risk of human infection through exposure to microorganisms in the aquatic environments [8,18–21].

In today's highly urbanized society, the sewerage system is highly developed [22]. Research has been conducted to find effective methods for predicting the concentration of AMRB in the STP influent, where wastewater is concentrated [23,24], and these methods have been supported by administrative agencies in Europe and the United States [25,26]. Previous research has evaluated a method for predicting the concentration of pharmaceuticals in the STP influent based on annual statistics of domestic production [26] and sales [25] of pharmaceuticals, such as prescription drugs (e.g., anti-inflammatory diclofenac, antihypertensive metoprolol, psychotropic carbamazepine, anticancer cyclophosphamide, and antimicrobial sulfamethoxazole), as well as over-the-counter drugs (OTCs). The order of magnitude has been mostly in agreement, although there are differences of approximately 10 times between the two types of predicted concentrations and the measured concentrations [27–32]. However, statistics on pharmaceutical use based on prescriptions and receipts on a nationwide scale are still in the process of being developed, even from a global perspective, and in many cases, there are no consolidated statistics, or they are not publicly published from the viewpoint of personal information; therefore, few studies exist, and much remains unknown [33]. Furthermore, previous studies have mainly investigated OTC drugs such as antipyretic analgesics, and further studies are essential for a comprehensive understanding of antimicrobials in the aquatic environments.

In Japan, two types of statistics on pharmaceutical use are available thus far. One type is statistics on annual sales volumes of pharmaceuticals [34], as provided by private businesses as part of market research, and the other type is statistics on annual shipping volumes of pharmaceuticals available on the market [35], as surveyed by the government. These two statistics are compiled as aggregate figures for all of Japan. In 2016, the Ministry of Health, Labour and Welfare released the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB), which is a collection of information on medical receipts and specific health examinations by prefecture, excluding personal information [36]. Antimicrobials are exclusively used as prescription drugs, and their use as OTC drugs is prohibited in Japan. Therefore, it is possible to accurately grasp the actual use of antimicrobials, excluding those purchased without insurance coverage [37,38]. Under these situations, by applying the NDB database, it may be possible to predict the concentration of antimicrobials in the environment with a high degree of accuracy, taking into account the actual conditions of use. Nevertheless, to our knowledge, no study has yet predicted concentrations on the basis of the volumes of antimicrobials prescribed; moreover, the correspondence between predicted and measured concentrations is mostly unknown.

The aim of this study is, therefore, to evaluate the effectiveness of the estimation method for predicting antimicrobial concentrations in wastewater by assessing the estimation's correspondence with the measured values obtained in the survey. This paper consists of the following structure. First, a seasonal, year-round monitoring survey was conducted for antimicrobials detected in influent from a STP located in urban areas of Japan. Next,

the amount of antimicrobials used in the study area during the period studied was estimated using three statistics: domestic annual sales, shipping, and NDB statistics, and then the predicted concentration of antimicrobials in the STP influent from each amount used were calculated. Finally, the correspondence between the measured values obtained in the survey and the predicted values estimated from the amount used was verified, and the effectiveness of the method as a method for predicting the environmental impact associated with antimicrobial use was evaluated.

## 2. Materials and Methods

### 2.1. Microbes and Reagents

A total of 10 antimicrobials were surveyed on the basis of the previous report of concentration levels and frequencies of detection in hospital effluent, sewage, and river water reported both in Japan and around the world, and antimicrobial use in Japan as well [15,39,40]. The detailed names of the target antimicrobials are ampicillin (APL), cefdinir (CDN), cefpodoxime proxetil (CPXP), ciprofloxacin (CFX), clarithromycin (CTM), doxycycline (DCL), levofloxacin (LFX), minocycline (MCL), tetracycline (TCL), and vancomycin (VMC). The physicochemical properties and ratios of excretion as unchanged after ingestion are summarized in Table 1. All analytical standards were of high purity (>98%). Individual standard stock solutions at 10 mg/L were prepared in methanol and stored at  $-20\text{ }^{\circ}\text{C}$ . All aqueous solutions were prepared with ultrapure Milli-Q water (18.2 M $\Omega$ -cm; Millipore Sigma, Watford, UK). Liquid chromatography–mass spectrometry (LC–MS)-grade methanol (>99%), formic acid (>99%), and acetone (>99%), analytical grade hydrochloric acid (>37%), ammonia (>30%), and ascorbic acid (>99%) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

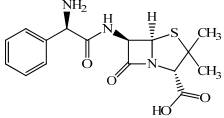
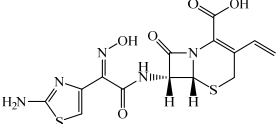
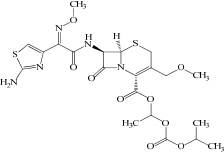
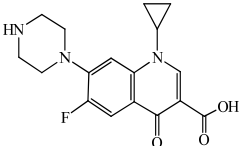
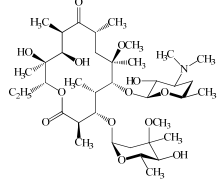
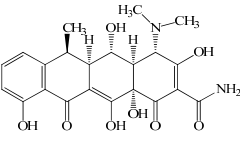
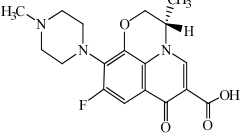
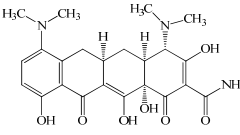
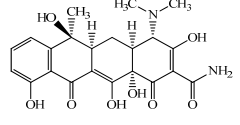
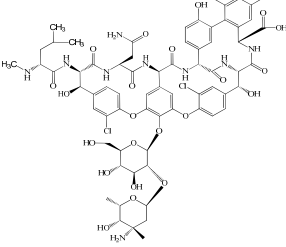
### 2.2. Sampling

STP influent was collected at a representative STP located in the Yodo River basin of Japan, which has a population of 17 million (14% of the Japanese population) [41,42]. At the time of this study, the STP disposed of municipal wastewater from 369,000 people, and the annual mean flow rate was  $189,730 \pm 5000\text{ m}^3/\text{day}$ . The wastewater in the STP was treated with conventional activated sludge and then chlorine disinfection was applied. Sewage sludge produced in the treatment process is dewatered in a centrifuge and incinerated for disposal.

Sampling was conducted during 2019–2021: October 2019, September 2020, January 2021, and March 2021. This sampling frequency was on the basis of previous reports similar to the present research [31,32,43]. Identical manual sampling was adopted at a fixed sampling frequency because a composite sampler could not be used at the STP. Sampling was conducted on rain-free days, and no rainfall greater than 1 mm was observed during the two days before the sampling day [44].

A stainless-steel pail sampler was used to collect 100-mL samples of STP influent. Water samples were placed in sterilized glass bottle samples, and ascorbic acid (1 g/L) was immediately added as a preservative for antimicrobials [7,45]. All water samples were immediately transported to the laboratory in a cooler box (within 1 to 2 h), stored at  $4\text{ }^{\circ}\text{C}$  in darkness, and processed within 24 h.

**Table 1.** Properties of the target antimicrobials examined [46–54].

Antimicrobials	Molecular Formula	Molecular Mass (g/mol)	Structure	Excretion Rate (%)
Ampicillin (APL)	$C_{16}H_{19}N_3O_4S$	349.4		71 [46]
Cefdinir (CDN)	$C_{14}H_{13}N_5O_5S_2$	395.4		31 [47]
Cefpodoxime proxetil (CPXP)	$C_{21}H_{27}N_5O_9S_2$	557.6		50 [48]
Ciprofloxacin (CFX)	$C_{17}H_{18}FN_3O_3$	331.3		50 [49]
Clarithromycin (CTM)	$C_{38}H_{69}NO_{13}$	748.0		24 [50]
Doxycycline (DCL)	$C_{22}H_{24}N_2O_8$	444.4		80 [51]
Levofloxacin (LFX)	$C_{18}H_{20}FN_3O_4$	361.4		83 [52]
Minocycline (MCL)	$C_{23}H_{27}N_3O_7$	457.5		36 [51]
Tetracycline (TCL)	$C_{22}H_{24}N_2O_8$	444.4		60 [53]
Vancomycin (VMC)	$C_{66}H_{75}Cl_2N_9O_{24}$	1449.3		85 [54]

### 2.3. Analytical Procedures for Antimicrobials

The concentrations of target antimicrobials in the wastewater was determined by using a combination of solid phase extraction (SPE) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS), as described previously [7].

Briefly, 10 mL of STP influent was filtered through a glass-fibre filter (GF/B, 1- $\mu$ m pore size, Whatman, Maidstone, UK). A known amount of each antimicrobial was spiked into two of the sample solutions for analysis to make a final concentration of 200 ng/L to account for matrix effects and loss during sample extraction [55]. The solutions were then subjected to two different preconditioned SPE cartridges (OASIS HLB, 200 mg; Waters Corp., Milford, MA, USA) at a flow rate of 1 mL/min; the cartridges had been preconditioned for each sample by being washed first with 3 mL of methanol and then with 3 mL of Milli-Q water adjusted to pH 3 with 1 N HCl. Similarly, the two unspiked sample solutions were removed and processed as above. All cartridges were washed with 6 mL of Milli-Q water preadjusted to pH 3 and then dried by a vacuum pump. Finally, the adsorbed antimicrobials were separately eluted with 3 mL of acetone and 3 mL methanol for one, and for the other with 2 mL of 10% (*v/v*) formic acid in acetone, 2 mL of 10% (*v/v*) formic acid in methanol, and 2 mL of 5% ammonia-methanol (*v/v*).

Each combined eluted solution was evaporated mildly to dryness under a gentle stream of N<sub>2</sub> gas at 37 °C. The residue was solubilized in 200  $\mu$ L of a 90:10 (*v/v*) mixture of 0.1% formic acid solution in methanol, and 10  $\mu$ L of this solution was subjected for the UPLC system coupled to a tandem quadrupole mass spectrometer (TQD, Waters Corp.) equipped with an electrospray ionization source with positive and negative ion modes. Detailed information on the analytical parameters used for UPLC–MS/MS analysis is given in Table 2.

**Table 2.** LC–MS/MS parameters and validations of each antimicrobial.

Antimicrobials	Ionization Mode	Precursor Ion ( <i>m/z</i> )	Product Ion ( <i>m/z</i> )	Cone Voltage (V)	Collision Energy (eV)	Recovery (% (SD))	LOD (ng/L)	LOQ (ng/L)
Ampicillin (APL)	ESI+	350.2	<i>105.9, 192.0</i>	29	24	75 (33)	1.6	5.4
Cefdinir (CDN)	ESI+	369.2	<i>170.0, 227.0</i>	30	20	72 (15)	0.2	0.8
Cefpodoxime proxetil (CPXP)	ESI+	557.5	<i>409.8, 525.2</i>	30	18	89 (5)	1.2	3.9
Ciprofloxacin (CFX)	ESI+	332.2	<i>288.2, 314.2</i>	40	25	57 (3)	0.7	2.2
Clarithromycin (CTM)	ESI+	748.2	<i>316.6, 558.3</i>	38	18	74 (11)	0.5	1.6
Doxycycline (DCL)	ESI+	445.2	<i>428.3</i>	32	18	80 (9)	0.3	1.0
Levofloxacin (LFX)	ESI+	362.2	<i>261.2, 318.2</i>	40	21	52 (6)	0.3	1.0
Minocycline (MCL)	ESI+	458.3	<i>441.0</i>	36	21	106 (18)	0.3	1.0
Tetracycline (TCL)	ESI+	445.2	<i>409.9, 427.1</i>	28	20	70 (20)	0.4	1.3
Vancomycin (VMC)	ESI+	724.2	<i>82.9, 100.2</i>	17	18	110 (8)	0.6	1.9

Product ions in italics were used for quantification.

### 2.4. Method Validation

Six-point standard calibration curves were prepared for quantification ranging between 0.5 and 200 ng/mL. Individual linear calibration curves for each antimicrobial were obtained in the concentration range of 0.5 to 200 ng/mL ( $r^2 > 0.99$ ) by selecting the weighting factor of  $1/x$ . Quantification was performed by subtracting the blank data from the corresponding data given by the spiked sample solutions to account for matrix effects and losses during sample extraction [56,57]. The recovery rates of antimicrobials in the STP influent ranged from 52% to 110% (Table 2). These profiles were generally similar to those reported in previous reports of pharmaceuticals in river water and wastewater samples [39,58,59]. The limits of detection (LODs) and limits of quantification (LOQs) were calculated as the concentrations at signal-to-noise ratios of 3 and 10, respectively, according to the methods applied to residual pharmaceuticals in environmental water samples [59,60]. These values are also summarized in Table 2.

### 2.5. Prediction of the Concentration of Antimicrobials in the Targeted STP Influent

The annual sales, shipping, and NDB statistical data are summarized in Table 3. Concentrations of antimicrobials in the targeted STP influent ( $C_{(STP)in}$ ) can be calculated by using annual sales volume summarized as an aggregate for all of Japan. (Equation (1)), as described below based on the previously reported estimation [31], assumes that antimicrobials are consumed equally every day [61,62] and that antimicrobial use is proportional to the population in each prefecture nationwide [25,27].

$$C_{(STP)in} = \frac{D_{(Sales)total} \times E}{365} \times \frac{P_{STP}}{P_{Japan}} \times \frac{1}{W_{STP}} \times 10^9 \quad (1)$$

where  $D_{(Sales)total}$  is the distribution of antimicrobials based on the annual shipping volumes in Japan considering pharmaceutical price and content (kg/year) (Table 3),  $E$  is the excretion rate of the target antimicrobials after administration as unchanged form (%) (Table 1), 365 is the number of days per year,  $P_{STP}$  is the service population in the STP (number of persons) (369,000),  $P_{Japan}$  is the population of Japan (126,227,000 persons) [63],  $W_{STP}$  is the amount of influent water at the STP (189,730 m<sup>3</sup>/day) [64], and 10<sup>9</sup> is a factor for unit conversion from kg/m<sup>3</sup> to ng/L.

**Table 3.** Annual antimicrobial usage in the survey area [34–36].

Antimicrobials	Antimicrobial Usage (Mean (SD))		
	Sales * [34] (kg/year)	Shipping * [35] (kg/year)	NDB ** [36] (kg/year)
Ampicillin (APL)	3313 (562)	N.A.	182 (22)
Cefdinir (CDN)	2810 (181)	6707 (953)	410 (101)
Cefpodoxime proxetil (CPXP)	975 (276)	1588 (588)	130 (12)
Ciprofloxacin (CFX)	N.A.	1405 (241)	148 (13)
Clarithromycin (CTM)	11,592 (3970)	71,850 (15,086)	5499 (63)
Doxycycline (DCL)	N.A.	N.A.	93 (9)
Levofloxacin (LFX)	1683 (536)	32,548 (4441)	2665 (11)
Minocycline (MCL)	N.A.	4157 (905)	377 (36)
Tetracycline (TCL)	N.A.	N.A.	14 (6)
Vancomycin (VMC)	158 (25)	2905 (186)	169 (7)

\*: Antimicrobial usage in Japan, \*\*: Antimicrobial usage in the target area in this study, N.A.: Not available.

Concentrations of targeted antimicrobials in the targeted STP influent ( $C_{(STP)in}$ ) can be calculated by using annual shipping volumes summarized as an aggregate for all of Japan (kg/year) (Equation (2)) [31] and by assuming that all shipped antimicrobials are consumed equally every day [61,62] and that antimicrobial use is proportional to the population in each prefecture nationwide [25–28,30]:

$$C_{(STP)in} = \frac{D_{(Ship)total} \times E}{365} \times \frac{P_{STP}}{P_{Japan}} \times \frac{1}{W_{STP}} \times 10^9 \quad (2)$$

Furthermore, the concentrations of antimicrobials in the targeted STP influent ( $C_{(STP)in}$ ) can be calculated by using the annual NDB volumes summarized as prefecture-by-prefecture aggregate values (kg/year) (Equation (3)) and by assuming that all prescribed antimicrobials are consumed equally every day [37,61,62] and that antimicrobial use is proportional to the population in each prefecture nationwide [25–28,30]:

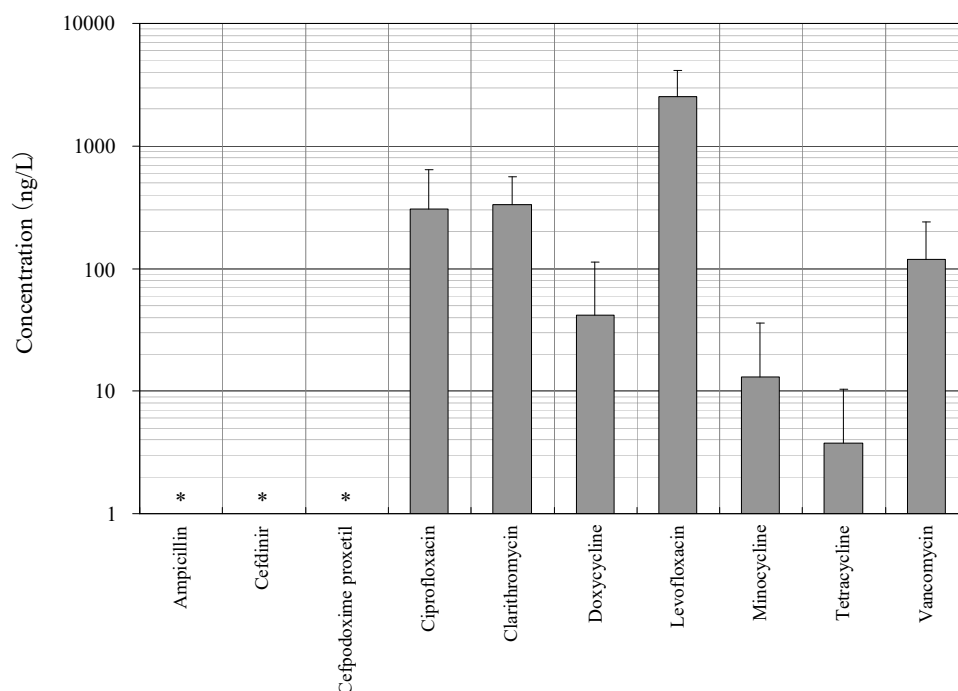
$$C_{(STP)in} = \frac{D_{(Receipt)prefecture} \times E}{365} \times \frac{P_{STP}}{P_{Prefecture}} \times \frac{1}{W_{STP}} \times 10^9 \quad (3)$$

where  $D_{(Receipt)prefecture}$  is the distribution of antimicrobials based on the annual NDB volumes in the prefectures (kg/year) (Table 3) and  $P_{Prefecture}$  is the population of the prefecture (8,842,523 persons) [63].

### 3. Results and Discussion

#### 3.1. Distribution of Concentrations of Antimicrobials in the STP Influent

Seven antimicrobials (CFX, CTM, DCL, LFX, MCL, TCL, and VMC) were detected at a wide range of concentrations from ng/L to  $\mu\text{g/L}$  levels (11 ng/L to a maximum of 4.3  $\mu\text{g/L}$ ) during the survey period in the STP influent (Figure 1). The detected concentrations of antimicrobials were similar to those previously reported [7,39,65]. The average detected concentrations of each antimicrobial were  $309 \pm 334$  ng/L (CFX),  $336 \pm 223$  ng/L (CTM),  $42 \pm 72$  ng/L (DCL),  $2536 \pm 1617$  ng/L (LFX),  $13 \pm 23$  ng/L (MCL),  $4 \pm 7$  ng/L (TCL), and  $118 \pm 120$  ng/L (VMC). These results indicated that the antimicrobials used in the present study were used constantly throughout the year, with less seasonal fluctuation in use. This trend was mostly reasonable when trends in clinical use [61] were considered. In addition, pharmaceuticals detected in the environment, including antimicrobial components, are persistent in wastewater treatment processes and/or in the river environments [66,67] and remain in aquatic environments for long periods of time [68,69], the environmental risk of their inflow into the aquatic environments was considered to be a concern [70,71]. On the other hand, APL, CDN, and CPXP were not detected throughout the survey period. The reason for the lack of detection of these antimicrobials in the STP influent was related to the report that these antimicrobials are  $\beta$ -lactam antimicrobials with the common  $\beta$ -lactam structure in the structural formula and are easily degraded in the environment, being attenuated in a few hours to a few days [69,72], and were thought to be degraded in the sewage system before entering the STP.



**Figure 1.** Measured concentrations of targeted antimicrobials in STP influents (\*: Not detected).

#### 3.2. Prediction of Annual Concentrations of Targeted Antimicrobials in the STP Influent

The estimated concentrations of each antimicrobial in the targeted STP influent on the basis of annual sales, shipping, and NDB volumes are shown in Table 4.

**Table 4.** Predicted concentrations of antimicrobials in the target STP influent.

Antimicrobials	Predicted Concentrations in the Targeted STP Influent (ng/L) (Mean (SD))		
	Sales	Shipping	NDB
Ampicillin (APL)	99 (17)	N.A.	78 (10)
Cefdinir (CDN)	37 (2.4)	87 (12)	77 (19)
Cefpodoxime proxetil (CPXP)	20 (5.8)	33 (12)	39 (3.4)
Ciprofloxacin (CFX)	N.A.	29 (5.1)	44 (4.2)
Clarithromycin (CTM)	117 (40)	724 (152)	790 (0.1)
Doxycycline (DCL)	N.A.	N.A.	48 (4.8)
Levofloxacin (LFX)	59 (19)	1134 (155)	1335 (4.9)
Minocycline (MCL)	N.A.	63 (14)	82 (7.9)
Tetracycline (TCL)	N.A.	N.A.	4.9 (1.8)
Vancomycin (VMC)	5.6 (0.9)	104 (6.6)	87 (4.0)

N.A.: Not available.

Concentrations calculated based on the shipping and NDB volumes were similar, and a significant difference ( $p < 0.05$ ) was not observed in estimated concentrations between the shipping volumes and NDB volumes. In contrast, a significant difference ( $p < 0.05$ ) was observed in estimated concentrations between the sales volumes and both shipping and NDB volumes; concentrations predicted on the basis of sales volumes were approximately two times lower than those predicted by using shipping and NDB volumes in the case of CDN and CPXP, seven times lower in the case of CTM, and approximately 20 times lower in the case of LFX and VMC. This difference probably occurred due to the statistics on shipping volumes and NDB volumes covering all pharmaceutical companies in Japan, but the statistics on sales volumes covered only those antimicrobials sold by major pharmaceutical companies, and targeted antimicrobials in this study are all sold by a large number of pharmaceutical companies as generic drugs [34]. These discrepancies in reporting are similar to those found in the previous studies [31,32].

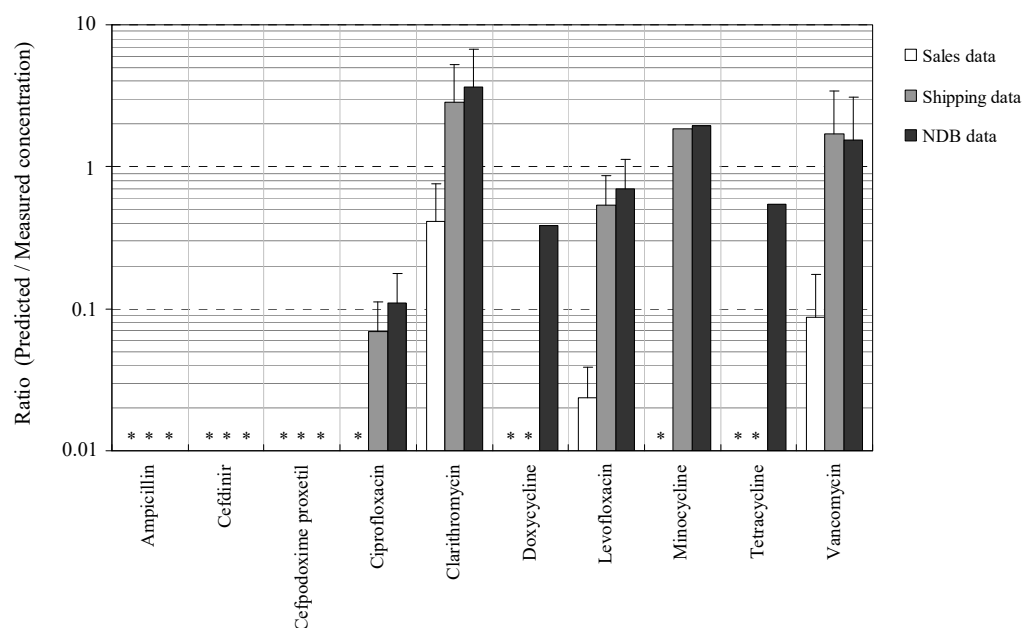
### 3.3. Relationships between Measured and Predicted Concentrations of Antimicrobials

The correspondence ratios between the measured concentrations as described in Section 3.1 and the predicted concentrations estimated on the basis of sales, shipping, and NDB volumes as described in Section 3.2 as a ratio of predicted concentration/measured concentration are summarized in Figure 2.

The correspondence ratio between the predicted concentration calculated on the basis of sales volume and the measured concentration was  $0.4 \pm 0.4$  for CTM,  $0.02 \pm 0.01$  for LFX, and  $0.1 \pm 0.1$  for VMC. The main reason for the differences in the predicted concentration and measured concentration based on the sales volume is probably the fact that CTM, LFX, and VMC are sold by many pharmaceutical companies as generic antimicrobials [34]. This trend supports the reports of a difference between NDB antimicrobial use and antimicrobial sales [38]. Interestingly, the orders of magnitude of the predicted and measured concentrations were generally in agreement for shipping and NDB volumes. The correspondence ratio between the predicted concentration calculated on the basis of shipping volume and the measured concentration was  $0.1 \pm 0.1$  for CFX,  $2.9 \pm 2.4$  for CTM,  $0.5 \pm 0.3$  for LFX, 1.9 for MCL, and  $1.7 \pm 1.7$  for VMC, and that on the basis of NDB volume and the measured concentration was  $0.1 \pm 0.1$  for CFX,  $3.7 \pm 3.1$  for CTM, 0.4 DCL,  $0.7 \pm 0.4$  for LFX, 1.9 for MCL, 0.6 for TCL, and  $1.6 \pm 1.6$  for VMC. The correspondence ratios generally agreed with the results in the present study, with previously reported results conducted in different regions based on the estimation from shipping volume (0.9 to 2.9 for CFX, 1.0 to 2.1 for CTM, and 18.3 for LFX [31,32]), although, in all cases, the effects of household disposal could not be excluded in the present estimations, as noted [73]. On the other hand, the fact that  $\beta$ -lactam antimicrobials APL, CDN, and CPXP were not detected in the effluent was



considered to be due to the deviation from the predicted concentration due to their easy attenuation in the environment, as described in Section 3.1.



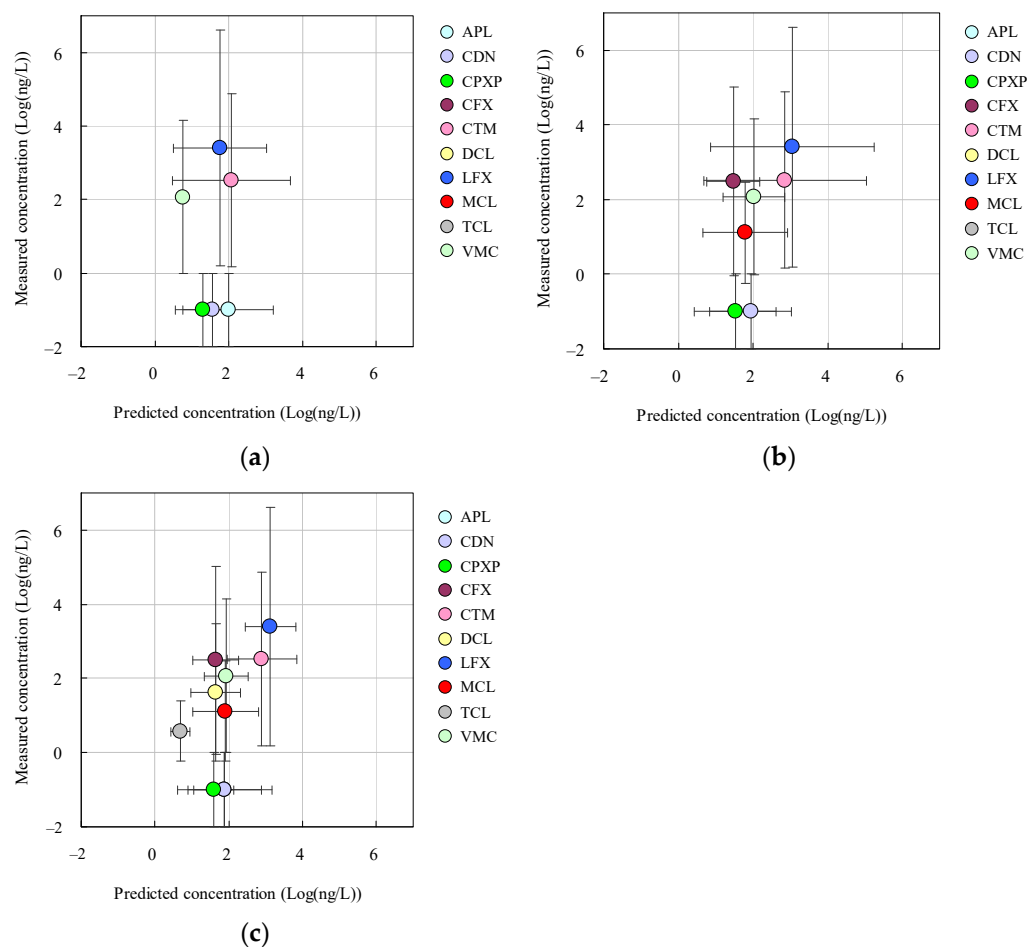
**Figure 2.** Correspondence ratios for predicted/measured concentration of target antimicrobials in STP influents (\*: Not detected).

The measured concentration tended to be approximately 10 times higher than the predicted concentration in CFX in this study, even in the estimation from shipping and NDB volume. The low predicted concentration of CFX may originate from veterinary and livestock fields as well as from human use. Practically, the use of antimicrobials in animals is approximately twice as high as that in humans [74,75], and this trend is similar worldwide [76]. These results suggest that it would be important to attempt to evaluate veterinary antimicrobials with regional characteristics in addition to those used for human use to implement of risk assessment and management of the impact of antimicrobials in the aquatic environments.

#### 3.4. Evaluation of Relationships between Concentrations of Antimicrobials Predicted by Using Sales, Shipping, and NDB Volumes

The correspondence ratios between the predicted concentrations based on the measured concentration and predicted concentrations on the basis of the sales, shipping, and NDB volumes are plotted against each other in Figure 3.

There was a positive correlation between the two (correlation coefficient  $r = 0.72$  for shipping volume and  $0.79$  for NDB volume). It is therefore possible to calculate predicted concentrations with better precision by using statistical data on shipping volumes and/or NDB volumes, taking into account the actual conditions of use. On the other hand, in Japan, information on the shipping volumes of new pharmaceuticals used in the forefront of clinical practice is not available publicly for reasons associated with the patents applied to the manufacture and sales of these pharmaceuticals [35]. Therefore, the availability of statistical information on NDB volumes may be irreplaceable in some specific cases. For this reason, for the evaluation of new pharmaceuticals, improving the correspondence between measured and predicted values is an issue that needs to be addressed in the future.



**Figure 3.** Relationship between correspondence ratios determined by using measured concentration values and the three types of predicted concentration values: (a) sales volume, (b) shipping volume, (c) NBD volume.

The present method developed for predicting the concentrations of antimicrobials in the STP influent is simple, accurate, and precise. By taking into consideration the removal rates of pharmaceuticals by wastewater treatment processes at STPs and the dilution and attenuation rates during the river water flow where the influents are to be discharged, the use of the method used in this study should improve the accuracy of concentration prediction approaches targeting aquatic environments [77–79].

To the best of our knowledge, this is the first report to show the results of an evaluation of the accuracy of and correspondence between predicted concentrations based on sales, shipping, NDB statistics and measured concentrations for antimicrobials in the STP influent. These findings will contribute to a comprehensive understanding of the environmental risks associated with the presence of antimicrobials in the aquatic environments. In the future, the method developed here should be useful for the priority screening of antimicrobials and for conducting environmental risk assessments of antimicrobials that are yet to be studied in the aquatic environments. Furthermore, the introduction of advanced water treatment systems, such as ozonation [80,81], adsorption [82,83], membrane [84,85], electrochemical [86,87], and peracetic acid [88,89] are considered to be addressed in removing large amounts of pollutant loads including antimicrobial-resistant bacteria into the aquatic environments.

#### 4. Limitations

The following limitations can be noted in this study. First, this study evaluated representative antimicrobials that are used in Japan and are considered to have higher con-

centrations detected in wastewater. Therefore, antimicrobials belonging to some medicinal classifications, such as sulfonamides [90] and aminoglycosides [91], were not investigated in this study, and for antimicrobials used for both human and animal use [92], there is a possibility that the discrepancy between the measured and predicted concentrations may be large, as mentioned in this report. Second, grab sampling was used for collecting wastewater at the STP in this study, and the concentration of antimicrobials in wastewater measured represents the concentration at a certain time of the day. Therefore, it would be possible that errors are due to diurnal variations caused by sampling methods compared to the continuous proportional sampling and flow proportional mode sampling [93,94].

Finally, the statistical aggregation of pharmaceutical use has limitations in that it does not include information on pharmaceuticals imported and purchased from abroad by individuals, as the regions where pharmaceuticals are prescribed may differ from the regions where they are actually used by patients, and it would also be affected by medication compliance, which assumes that all prescribed antimicrobials are taken [37,38]. Our results support the need for further, conclusive research by taking experimental, technical, regional customs, bias, and unknown factors into consideration.

## 5. Conclusions

The predicted concentrations of the representative antimicrobials in the STP influent of Japan by estimation from antimicrobial shipping and NDB volumes generally agreed with the measured concentrations with good accuracy. Additionally, although the concentrations predicted from the sales volumes were somewhat lower than those predicted from the shipping and NDB volumes, the orders of magnitude of the measured and predicted concentrations were roughly in agreement. These results indicate that the concentrations of antimicrobials in the STP influents can be estimated with high accuracy from two assets, antimicrobial shipping and NDB volumes. The fact that there is evidence that the use of antimicrobials has a direct impact on the environments is significant in terms of taking countermeasures for antimicrobials in the aquatic environments. Consideration of the removal rates during wastewater treatment processes at STPs and the dilution and attenuation rates in the receiving rivers should improve the accuracy of this method in predicting the concentrations of antimicrobials in river waters.

These findings highlight the effectiveness of the prediction of concentrations of antimicrobials in the STP influent. The importance of reducing the amount of antimicrobials before they are discharged into rivers was also evoked for maintaining both a clean environment and for human health. This study provides a new means of assessing the environmental risks associated with antimicrobials in aquatic environments.

**Author Contributions:** Conceptualization T.A. and T.H.; investigation: T.A. and T.N.; methodology: T.A., R.K. and N.M.; formal analysis: T.A., R.K. and N.M.; writing-original draft: T.A., T.N. and T.H.; writing-review and editing: T.A., T.N. and T.H.; supervision: T.A. and N.O.; funding acquisition: T.A.; project administration: T.A., T.N., N.O. and T.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Lake Biwa–Yodo River Water Quality Preservation Organization (Japan); the River Foundation (Japan); the Kurita Water and Environment Foundation (Japan); the Ministry of Education, Culture, Sports, Science and Technology of Japan (16K16218 and 20H02289); the Ministry of Health, Labor and Welfare of Japan (21HA1002); and the Japan Agency for Medical Research and Development (AMED) (JP22fk0108131) in the form of research grants and scholarships.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** We thank the staff of the STPs for providing water samples.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

## References

1. Nathan, C.; Cars, O. Antibiotic resistance—Problems, progress, and prospects. *N. Engl. J. Med.* **2014**, *371*, 1761–1763. [[CrossRef](#)] [[PubMed](#)]
2. Walsh, T.R. A One-Health approach to antimicrobial resistance. *Nature Microbiol.* **2018**, *3*, 854–855. [[CrossRef](#)] [[PubMed](#)]
3. Schages, L.; Wichern, F.; Kalscheuer, R.; Bockmühl, D. Winter is coming—Impact of temperature on the variation of beta-lactamase and *mcr* genes in a wastewater treatment plant. *Sci. Total Environ.* **2020**, *712*, 136499. [[CrossRef](#)] [[PubMed](#)]
4. Cahill, N.; O'Connor, L.; Mahon, B.; Varley, Á.; McGrath, E.; Ryan, P.; Cormican, M.; Brehony, C.; Jolley, K.A.; Maiden, M.C.; et al. Hospital effluent: A reservoir for carbapenemase-producing Enterobacterales? *Sci. Total Environ.* **2019**, *672*, 618–624. [[CrossRef](#)]
5. Paulus, G.K.; Hornstra, L.M.; Alygizakis, N.; Slobodnik, J.; Thomaidis, N.; Medema, G. The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. *Int. J. Hyg. Environ. Health* **2019**, *222*, 635–644. [[CrossRef](#)]
6. Hassoun-Kheir, N.; Stabholz, Y.; Kreft, J.U.; de la Cruz, R.; Romalde, J.L.; Nesme, J.; Sørensen, S.J.; Smets, B.F.; Graham, D.; Paul, M. Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. *Sci. Total Environ.* **2020**, *743*, 140804. [[CrossRef](#)]
7. Azuma, T.; Otomo, K.; Kunitou, M.; Shimizu, M.; Hosomaru, K.; Mikata, S.; Ishida, M.; Hisamatsu, K.; Yunoki, A.; Mino, Y.; et al. Environmental fate of pharmaceutical compounds and antimicrobial-resistant bacteria in hospital effluents, and contributions to pollutant loads in the surface waters in Japan. *Sci. Total Environ.* **2019**, *657*, 476–484. [[CrossRef](#)]
8. Zainab, S.M.; Junaid, M.; Xu, N.; Malik, R.N. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Res.* **2020**, *187*, 116455. [[CrossRef](#)]
9. Zheng, D.; Yin, G.; Liu, M.; Chen, C.; Jiang, Y.; Hou, L.; Zheng, Y. A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *Sci. Total Environ.* **2021**, *777*, 146009. [[CrossRef](#)]
10. Pazda, M.; Kumirska, J.; Stepnowski, P.; Mulkiewicz, E. Antibiotic resistance genes identified in wastewater treatment plant systems—A review. *Sci. Total Environ.* **2019**, *697*, 134023. [[CrossRef](#)]
11. Nguyen, A.Q.; Vu, H.P.; Nguyen, L.N.; Wang, Q.; Djordjevic, S.P.; Donner, E.; Yin, H.; Nghiem, L.D. Monitoring antibiotic resistance genes in wastewater treatment: Current strategies and future challenges. *Sci. Total Environ.* **2021**, *783*, 146964. [[CrossRef](#)] [[PubMed](#)]
12. Miłobedzka, A.; Ferreira, C.; Vaz-Moreira, I.; Calderón-Franco, D.; Gorecki, A.; Purkrtova, S.; Jan, B.; Dziewit, L.; Singleton, C.M.; Nielsen, P.H.; et al. Monitoring antibiotic resistance genes in wastewater environments: The challenges of filling a gap in the one-health cycle. *J. Hazard. Mater.* **2022**, *424*, 127407. [[CrossRef](#)] [[PubMed](#)]
13. Carvalho, I.T.; Santos, L. Antibiotics in the aquatic environments: A review of the European scenario. *Environ. Int.* **2016**, *94*, 736–757. [[CrossRef](#)] [[PubMed](#)]
14. Meyer, M.F.; Powers, S.M.; Hampton, S.E. An evidence synthesis of pharmaceuticals and personal care products (PPCPs) in the environment: Imbalances among compounds, sewage treatment techniques, and ecosystem types. *Environ. Sci. Technol.* **2019**, *53*, 12961–12973. [[CrossRef](#)]
15. Tran, N.H.; Reinhard, M.; Gin, K.Y.H. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions—A review. *Water Res.* **2018**, *133*, 182–207. [[CrossRef](#)]
16. Gwenzi, W.; Musiyiwa, K.; Mangori, L. Sources, behaviour and health risks of antimicrobial resistance genes in wastewaters: A hotspot reservoir. *J. Environ. Chem. Eng.* **2020**, *8*, 102220. [[CrossRef](#)]
17. Gogoi, A.; Mazumder, P.; Tyagi, V.K.; Tushara Chaminda, G.G.; An, A.K.; Kumar, M. Occurrence and fate of emerging contaminants in water environment: A review. *Groundw. Sustain. Develop.* **2018**, *6*, 169–180. [[CrossRef](#)]
18. Bengtsson-Palme, J.; Larsson, D.G.J. Antibiotic resistance genes in the environment: Prioritizing risks. *Nat. Rev. Microbiol.* **2015**, *13*, 396. [[CrossRef](#)]
19. Kovalakova, P.; Cizmas, L.; McDonald, T.J.; Marsalek, B.; Feng, M.; Sharma, V.K. Occurrence and toxicity of antibiotics in the aquatic environment: A review. *Chemosphere* **2020**, *251*, 126351. [[CrossRef](#)]
20. Zhuang, M.; Achmon, Y.; Cao, Y.; Liang, X.; Chen, L.; Wang, H.; Siame, B.A.; Leung, K.Y. Distribution of antibiotic resistance genes in the environment. *Environ. Pollut.* **2021**, *285*, 117402. [[CrossRef](#)]
21. Ma, L.; Yang, H.; Guan, L.; Liu, X.; Zhang, T. Risks of antibiotic resistance genes and antimicrobial resistance under chlorination disinfection with public health concerns. *Environ. Int.* **2022**, *158*, 106978. [[CrossRef](#)] [[PubMed](#)]
22. Tao, Z.; Chen, P.; Cui, N.; Lin, X.; Ji, F.; Liu, Y.; Xiong, P.; Zhang, L.; Xu, Q.; Song, Y.; et al. Detection of enteroviruses in urban sewage by next generation sequencing and its application in environmental surveillance. *Sci. Total Environ.* **2020**, *728*, 138818. [[CrossRef](#)] [[PubMed](#)]
23. Hendriksen, R.S.; Munk, P.; Njage, P.; van Bunnik, B.; McNally, L.; Lukjancenko, O.; Röder, T.; Nieuwenhuijse, D.; Pedersen, S.K.; Kjeldgaard, J.; et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* **2019**, *10*, 1124. [[CrossRef](#)]
24. Aarestrup, F.M.; Woolhouse, M.E.J. Using sewage for surveillance of antimicrobial resistance. *Science* **2020**, *367*, 630–632. [[CrossRef](#)] [[PubMed](#)]
25. FDA (U.S. Department of Health and Human Services Food and Drug Administration). *Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications*; FDA: Washington, DC, USA, 1998; pp. 1–39.

26. EMEA (European Medicines Agency). *Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use*; EMEA: Amsterdam, The Netherlands, 2006; pp. 1–12.
27. Tauxe-Wuersch, A.; De Alencastro, L.F.; Grandjean, D.; Tarradellas, J. Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. *Water Res.* **2005**, *39*, 1761–1772. [[CrossRef](#)]
28. Carballa, M.; Omil, F.; Lema, J.M. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. *Chemosphere* **2008**, *72*, 1118–1123. [[CrossRef](#)] [[PubMed](#)]
29. Besse, J.P.; Latour, J.F.; Garric, J. Anticancer drugs in surface waters: What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs? *Environ. Int.* **2012**, *39*, 73–86. [[CrossRef](#)]
30. Oosterhuis, M.; Sacher, F.; ter Laak, T.L. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. *Sci. Total Environ.* **2013**, *442*, 380–388. [[CrossRef](#)]
31. Azuma, T.; Nakada, N.; Yamashita, N.; Tanaka, H. Evaluation of concentrations of pharmaceuticals detected in sewage influents in Japan by using annual shipping and sales data. *Chemosphere* **2015**, *138*, 770–776. [[CrossRef](#)]
32. He, K.; Borthwick, A.G.; Lin, Y.; Li, Y.; Fu, J.; Wong, Y.; Liu, W. Sale-based estimation of pharmaceutical concentrations and associated environmental risk in the Japanese wastewater system. *Environ. Int.* **2020**, *139*, 105690. [[CrossRef](#)]
33. Gao, J.; Tschärke, B.J.; Choi, P.M.; O'Brien, J.W.; Boogaerts, T.; Jiang, H.; Yang, M.; Hollingworth, S.A.; Thai, P.K. Using prescription and wastewater data to estimate the correction factors of atenolol, carbamazepine, and naproxen for wastewater-based epidemiology applications. *Environ. Sci. Technol.* **2021**, *55*, 7551–7560. [[CrossRef](#)] [[PubMed](#)]
34. Jiho. *Yakuji Handbook*; Jiho: Tokyo, Japan, 2021; ISBN 978-4840753494. (In Japanese)
35. Ministry of Health Labour and Welfare, Japan. Annual Report on Statistics of Production by Pharmaceutical Industry in 2020. Available online: <https://www.mhlw.go.jp/topics/yakuji/2020/nenpo/> (accessed on 7 March 2022). (In Japanese)
36. Ministry of Health Labour and Welfare, Japan. National Database of Health Insurance Claims and Specific Health Check-ups of Japan (NDB). Available online: <https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000177182.html> (accessed on 7 March 2022). (In Japanese)
37. Koizumi, R.; Kusama, Y.; Muraki, Y.; Ishikane, M.; Yamasaki, D.; Tanabe, M.; Ohmagari, N. Effect of population inflow and outflow between rural and urban areas on regional antimicrobial use surveillance. *PLoS ONE* **2021**, *16*, e0248338. [[CrossRef](#)]
38. Kusama, Y.; Muraki, Y.; Tanaka, C.; Koizumi, R.; Ishikane, M.; Yamasaki, D.; Tanabe, M.; Ohmagari, N. Characteristics and limitations of national antimicrobial surveillance according to sales and claims data. *PLoS ONE* **2021**, *16*, e0251299. [[CrossRef](#)] [[PubMed](#)]
39. Oliveira, T.S.; Murphy, M.; Mendola, N.; Wong, V.; Carlson, D.; Waring, L. Characterization of pharmaceuticals and personal care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. *Sci. Total Environ.* **2015**, *518–519*, 459–478. [[CrossRef](#)] [[PubMed](#)]
40. Azuma, T.; Arima, N.; Tsukada, A.; Hirami, S.; Matsuoka, R.; Moriwake, R.; Ishiuchi, H.; Inoyama, T.; Teranishi, Y.; Yamaoka, M.; et al. Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. *Sci. Total Environ.* **2016**, *548–549*, 189–197. [[CrossRef](#)]
41. Lake Biwa-Yodo River Water Quality Preservation Organization, Japan. *Lake Biwa-Yodo River Water Quality Preservation Organization (BYQ) Report on Water Environment in Biwa Lake-Yodo River System 2019*; BYQ: Osaka, Japan, 2021; pp. 1–90. (In Japanese)
42. Ministry of Health Labour and Welfare, Japan. *Vital Statistics in Japan*; Ministry of Health Labour and Welfare: Tokyo, Japan, 2021; pp. 1–102. (In Japanese)
43. Kumar, V.; Nakada, N.; Yamashita, N.; Johnson, A.C.; Tanaka, H. How seasonality affects the flow of estrogens and their conjugates in one of Japan's most populous catchments. *Environ. Pollut.* **2011**, *159*, 2906–2912. [[CrossRef](#)]
44. Japan Meteorological Agency 2021. Weather Statistics. Available online: <http://www.jma.go.jp/jma/index.html> (accessed on 7 March 2022).
45. Kumar, V.; Nakada, N.; Yasojima, M.; Yamashita, N.; Johnson, A.C.; Tanaka, H. Rapid determination of free and conjugated estrogen in different water matrices by liquid chromatography-tandem mass spectrometry. *Chemosphere* **2009**, *77*, 1440–1446. [[CrossRef](#)]
46. Lorenzen, J.M.; Broll, M.; Kaefer, V.; Burhenne, H.; Hafer, C.; Clajus, C.; Knitsch, W.; Burkhardt, O.; Kielstein, J.T. Pharmacokinetics of ampicillin/sulbactam in critically III patients with acute kidney injury undergoing extended dialysis. *Clin. J. American Soc. Nephrol.* **2012**, *7*, 385. [[CrossRef](#)]
47. Hishida, A.; Ohishi, K.; Nagashima, S.; Kanamaru, M.; Obara, M.; Kitada, A. Pharmacokinetic study of an oral cephalosporin, cefdinir, in hemodialysis patients. *Antimicrob. Agents Chemother.* **1998**, *42*, 1718–1721. [[CrossRef](#)]
48. Todd, W.M. Cefpodoxime proxetil: A comprehensive review. *Int. J. Antimicrob. Agents* **1994**, *4*, 37–62. [[CrossRef](#)]
49. Lee, E.; Williams, K. Clinical pharmacokinetics of ciprofloxacin. *Clin. Pharmacokinet.* **1990**, *19*, 503. [[PubMed](#)]
50. Rodvold, K.A. Clinical pharmacokinetics of clarithromycin. *Clin. Pharmacokinet.* **1999**, *37*, 385–398. [[CrossRef](#)] [[PubMed](#)]
51. Saivin, S.; Houin, G. Clinical pharmacokinetics of doxycycline and minocycline. *Clin. Pharmacokinet.* **1988**, *15*, 355–366. [[CrossRef](#)] [[PubMed](#)]
52. Almeida, A.; Duarte, S.; Nunes, R.; Rocha, H.; Pena, A.; Meisel, L. Human and veterinary antibiotics used in Portugal—A ranking for ecosurveillance. *Toxics* **2014**, *2*, 188. [[CrossRef](#)]
53. Fisher, P.; House, F.; Inns, P.; Morrison, P.; Rogers, H.; Bradbrook, I. Effect of cimetidine on the absorption of orally administered tetracycline. *Br. J. Clin. Pharmacol.* **1980**, *9*, 153–158. [[CrossRef](#)]

54. Matzke, G.R.; Zhanel, G.G.; Guay, D.R.P. Clinical pharmacokinetics of vancomycin. *Clin. Pharmacokinet.* **1986**, *11*, 257–282. [[CrossRef](#)]
55. de Jesus Gaffney, V.; Almeida, C.M.M.; Rodrigues, A.; Ferreira, E.; Benoliel, M.J.; Cardoso, V.V. Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. *Water Res.* **2015**, *72*, 199–208. [[CrossRef](#)]
56. Prasse, C.; Schlüsener, M.P.; Schulz, R.; Ternes, T.A. Antiviral drugs in wastewater and surface waters: A new pharmaceutical class of environmental relevance? *Environ. Sci. Technol.* **2010**, *44*, 1728–1735. [[CrossRef](#)]
57. Azuma, T.; Ishiuchi, H.; Inoyama, T.; Teranishi, Y.; Yamaoka, M.; Sato, T.; Mino, Y. Occurrence and fate of selected anticancer, antimicrobial, and psychotropic pharmaceuticals in an urban river in a subcatchment of the Yodo River Basin, Japan. *Environ. Sci. Pollut. Res.* **2015**, *22*, 18676–18686. [[CrossRef](#)]
58. Grabic, R.; Fick, J.; Lindberg, R.H.; Fedorova, G.; Tysklind, M. Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. *Talanta* **2012**, *100*, 183–195. [[CrossRef](#)]
59. Petrović, M.; Škrbić, B.; Živančev, J.; Ferrando-Climent, L.; Barcelo, D. Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole-linear ion trap in different types of water in Serbia. *Sci. Total Environ.* **2014**, *468–469*, 415–428. [[CrossRef](#)] [[PubMed](#)]
60. Schlüsener, M.P.; Hardenbicker, P.; Nilson, E.; Schulz, M.; Viergutz, C.; Ternes, T.A. Occurrence of venlafaxine, other antidepressants and selected metabolites in the rhine catchment in the face of climate change. *Environ. Pollut.* **2015**, *196*, 247–256. [[CrossRef](#)] [[PubMed](#)]
61. Sugawara, T.; Ohkusa, Y.; Gu, Y.; Taniguchi, K.; Okabe, N. Estimation of amount of antimicrobials used by pharmacy surveillance. *Jpn. J. Infect. Prevent. Control* **2012**, *27*, 195–198. [[CrossRef](#)]
62. Gu, Y.; Fujitomo, Y.; Ohmagari, N. Outcomes and future prospect of Japan’s National Action Plan on antimicrobial resistance (2016–2020). *Antibiotics* **2021**, *10*, 1293. [[CrossRef](#)] [[PubMed](#)]
63. Statistics Bureau Japan. *Statistical Handbook of Japan 2021*; Ministry of Health Labour and Welfare, Japan: Tokyo, Japan, 2021; pp. 1–197. ISBN 978-4-8223-4129-9.
64. Japan Sewage Works Association. *Statistics of Sewerage*; Japan Sewage Works Association: Tokyo, Japan, 2021. (In Japanese)
65. Singh, R.R.; Angeles, L.F.; Butryn, D.M.; Metch, J.W.; Garner, E.; Vikesland, P.J.; Aga, D.S. Towards a harmonized method for the global reconnaissance of multi-class antimicrobials and other pharmaceuticals in wastewater and receiving surface waters. *Environ. Int.* **2019**, *124*, 361–369. [[CrossRef](#)]
66. Couto, C.F.; Lange, L.C.; Amaral, M.C.S. Occurrence, fate and removal of pharmaceutically active compounds (PhACs) in water and wastewater treatment plants—A review. *J. Water Proc. Eng.* **2019**, *32*, 100927. [[CrossRef](#)]
67. Adeleye, A.S.; Xue, J.; Zhao, Y.; Taylor, A.A.; Zenobio, J.E.; Sun, Y.; Han, Z.; Salawu, O.A.; Zhu, Y. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. *J. Hazard. Mater.* **2022**, *424*, 127284. [[CrossRef](#)]
68. Han, S.; Li, X.; Huang, H.; Wang, T.; Wang, Z.; Fu, X.; Zhou, Z.; Du, P.; Li, X. Simultaneous determination of seven antibiotics and five of their metabolites in municipal wastewater and evaluation of their stability under laboratory conditions. *Int. J. Environ. Res. Public Health* **2021**, *18*, 10640. [[CrossRef](#)]
69. Robles-Jimenez, L.E.; Aranda-Aguirre, E.; Castelan-Ortega, O.A.; Shettino-Bermudez, B.S.; Ortiz-Salinas, R.; Miranda, M.; Li, X.; Angeles-Hernandez, J.C.; Vargas-Bello-Pérez, E.; Gonzalez-Ronquillo, M. Worldwide traceability of antibiotic residues from livestock in wastewater and soil: A systematic review. *Animals* **2022**, *12*, 60. [[CrossRef](#)]
70. Chaturvedi, P.; Shukla, P.; Giri, B.S.; Chowdhary, P.; Chandra, R.; Gupta, P.; Pandey, A. Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. *Environ. Res.* **2021**, *194*, 110664. [[CrossRef](#)]
71. Wang, X.; Lin, Y.; Zheng, Y.; Meng, F. Antibiotics in mariculture systems: A review of occurrence, environmental behavior, and ecological effects. *Environ. Pollut.* **2022**, *293*, 118541. [[CrossRef](#)] [[PubMed](#)]
72. Lima, L.M.; Silva, B.N.M.d.; Barbosa, G.; Barreiro, E.J.  $\beta$ -lactam antibiotics: An overview from a medicinal chemistry perspective. *Eur. J. Med. Chem.* **2020**, *208*, 112829. [[CrossRef](#)] [[PubMed](#)]
73. Bound, J.P.; Voulvoulis, N. Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. *Environ. Health Perspect.* **2005**, *113*, 1705–1711. [[CrossRef](#)] [[PubMed](#)]
74. Shiozaki, Y. The action for the antimicrobial resistance issue in Japan. *J. Jpn. Assoc. Infect. Dis.* **2018**, *91*, 915–923.
75. Tamura, Y. Dissemination of antimicrobial-resistant bacteria based on the one health concept. *Jpn. J. Chemother.* **2018**, *66*, 715–728.
76. Tasho, R.P.; Cho, J.Y. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review. *Sci. Total Environ.* **2016**, *563–564*, 366–376. [[CrossRef](#)] [[PubMed](#)]
77. Verlicchi, P.; Al Aukidy, M.; Jelic, A.; Petrović, M.; Barceló, D. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: A case study of a catchment area in the Po Valley (Italy). *Sci. Total Environ.* **2014**, *470–471*, 844–854. [[CrossRef](#)]
78. Comber, S.; Gardner, M.; Sörme, P.; Ellor, B. The removal of pharmaceuticals during wastewater treatment: Can it be predicted accurately? *Sci. Total Environ.* **2019**, *676*, 222–230. [[CrossRef](#)] [[PubMed](#)]
79. Kuroda, K.; Li, C.; Dhangar, K.; Kumar, M. Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. *Sci. Total Environ.* **2021**, *776*, 145740. [[CrossRef](#)]

80. Beltrán, F.J.; Rey, A. Free radical and direct ozone reaction competition to remove priority and pharmaceutical water contaminants with single and hydrogen peroxide ozonation systems. *Ozone Sci. Eng.* **2018**, *40*, 251–265. [[CrossRef](#)]
81. Kharel, S.; Stapf, M.; Miehe, U.; Ekblad, M.; Cimbritz, M.; Falås, P.; Nilsson, J.; Sehlén, R.; Bester, K. Ozone dose dependent formation and removal of ozonation products of pharmaceuticals in pilot and full-scale municipal wastewater treatment plants. *Sci. Total Environ.* **2020**, *731*, 139064. [[CrossRef](#)] [[PubMed](#)]
82. Mangla, D.; Annu; Sharma, A.; Ikram, S. Critical review on adsorptive removal of antibiotics: Present situation, challenges and future perspective. *J. Hazard. Mater.* **2022**, *425*, 127946. [[CrossRef](#)] [[PubMed](#)]
83. Natarajan, R.; Saikia, K.; Ponnusamy, S.K.; Rathankumar, A.K.; Rajendran, D.S.; Venkataraman, S.; Tannani, D.B.; Arvind, V.; Somanna, T.; Banerjee, K.; et al. Understanding the factors affecting adsorption of pharmaceuticals on different adsorbents—A critical literature update. *Chemosphere* **2022**, *287*, 131958. [[CrossRef](#)] [[PubMed](#)]
84. Leiviskä, T.; Risteele, S. Analysis of pharmaceuticals, hormones and bacterial communities in a municipal wastewater treatment plant—Comparison of parallel full-scale membrane bioreactor and activated sludge systems. *Environ. Pollut.* **2022**, *292*, 118433. [[CrossRef](#)]
85. Sengar, A.; Vijayanandan, A. Effects of pharmaceuticals on membrane bioreactor: Review on membrane fouling mechanisms and fouling control strategies. *Sci. Total Environ.* **2022**, *808*, 152132. [[CrossRef](#)]
86. Chen, M.; Ren, L.; Qi, K.; Li, Q.; Lai, M.; Li, Y.; Li, X.; Wang, Z. Enhanced removal of pharmaceuticals and personal care products from real municipal wastewater using an electrochemical membrane bioreactor. *Bioresour. Technol.* **2020**, *311*, 123579. [[CrossRef](#)]
87. Zhang, J.; Zhou, Y.; Yao, B.; Yang, J.; Zhi, D. Current progress in electrochemical anodic-oxidation of pharmaceuticals: Mechanisms, influencing factors, and new technique. *J. Hazard. Mater.* **2021**, *418*, 126313. [[CrossRef](#)]
88. Cai, M.; Sun, P.; Zhang, L.; Huang, C.H. UV/peracetic acid for degradation of pharmaceuticals and reactive species evaluation. *Environ. Sci. Technol.* **2017**, *51*, 14217–14224. [[CrossRef](#)]
89. Dong, S.; Liu, Y.; Feng, L.; Zhang, L. Oxidation of pyrazolone pharmaceuticals by peracetic acid: Kinetics, mechanism and genetic toxicity variations. *Chemosphere* **2022**, *291*, 132947. [[CrossRef](#)]
90. Duan, W.; Cui, H.; Jia, X.; Huang, X. Occurrence and ecotoxicity of sulfonamides in the aquatic environment: A review. *Sci. Total Environ.* **2022**, *820*, 153178. [[CrossRef](#)]
91. Guironnet, A.; Sanchez-Cid, C.; Vogel, T.M.; Wiest, L.; Vulliet, E. Aminoglycosides analysis optimization using ion pairing liquid chromatography coupled to tandem mass spectrometry and application on wastewater samples. *J. Chromatogr. A* **2021**, *1651*, 462133. [[CrossRef](#)] [[PubMed](#)]
92. Kim, C.; Ryu, H.D.; Chung, E.G.; Kim, Y.; Lee, J.K. A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry. *J. Environ. Manag.* **2018**, *217*, 629–645. [[CrossRef](#)] [[PubMed](#)]
93. Gehring, T.; Deineko, E.; Hobus, I.; Kolisch, G.; Lübken, M.; Wichern, M. Effect of sewage sampling frequency on determination of design parameters for municipal wastewater treatment plants. *Water Sci. Technol.* **2020**, *84*, 284–292. [[CrossRef](#)] [[PubMed](#)]
94. Cristóvão, M.B.; Bento-Silva, A.; Bronze, M.R.; Crespo, J.G.; Pereira, V.J. Detection of anticancer drugs in wastewater effluents: Grab versus passive sampling. *Sci. Total Environ.* **2021**, *786*, 147477. [[CrossRef](#)]