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Antibiotics in Wastewater Treatment Plants in Tangshan: Perspectives on Temporal Variation, Residents' Use and Ecological Risk Assessment

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Abstract: In 2023, this study monitored nine types of antibiotics in the influent and effluent of wastewater treatment plants (WWTPs) in the urban and suburban areas of Tangshan. The total antibiotics concentration detected in influent WWTPs was highest in winter, followed by spring, summer, and autumn. The antibiotics concentration in influent and effluent urban WWTPs was higher than that in the suburban WWTPs in spring, summer, and winter, while the trend was reversed in autumn. Roxithromycin and oxytetracycline had a risk quotient (RQ) value of ≥ 0.1 in the effluent of WWTPs in winter, indicating that they are medium-risk antibiotics that pose a risk to the aquatic ecosystem after discharge. In the study area, the per capita pollution load of antibiotics was highest in spring, summer, and autumn for sulfamethoxazole, while it was highest in winter for ofloxacin. In the urban area, the use of roxithromycin, sulfamethoxazole, sulfamethoxazole, and ofloxacin was highest in spring, summer, autumn, and winter, respectively, while in suburban areas, the use of sulfamethoxazole, norfloxacin, sulfamethoxazole, and ofloxacin was highest during the same period. The use of antibiotics in the urban area was one order of magnitude higher than that in suburban areas, indicating a possible overuse of antibiotics in urban environments.

Keywords: antibiotics; wastewater treatment plant; temporal variation; use; ecological risk

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1. Introduction

Antibiotics play a crucial role in human health and are usually prioritized in drug management [1–3]. However, due to non-compliance with the principles of using antibiotics, there have been cases of inappropriate use and other forms of antibiotic abuse [4–6]. Currently, the misuse of antibiotics is a pressing public health and ecological security issue across various sectors, including medical health, food hygiene, livestock and poultry breeding, and ecological governance, not only in China but also globally [7–9]. China produces about 210,000 tons of antibiotic raw materials every year. Excluding the export of raw materials (about 30,000 tons), the remaining 180,000 tons are used domestically (including medical and agricultural use), with an annual per capita consumption of about 138 g [10]. As veterinary antibiotics, tetracycline antibiotics are the most commonly used, accounting for 40.5% of the total, followed by sulfonamides and macrolides. Quinolones are highly used in hospitals due to the high incidence of respiratory tract infections and mycoplasmal pneumonia in spring, autumn, and winter. Therefore, it is expected that antibiotics are widely present in urban sewage and agricultural wastewater, entering the water ecosystem through various pathways [11–13]. Due to the low metabolic rate of biological organisms against antibiotics, a large amount of antibiotics is excreted with urine and feces, collected by sewage networks, and entered WWTPs.

At present, the main purpose of WWTPs is to remove suspended solids, COD (chemical oxygen demand), nitrogen, phosphorus, and other substances in sewage, but they generally do not have the ability to efficiently remove antibiotics, resulting in high antibiotic concentrations in effluent wastewater [13,14]. It should be noted that sewage treatment technologies like advanced chemical oxidation, chemical precipitation, ultrafiltration, nanofiltration, and ion exchange are not effective at removing antibiotics and may be better suited for industrial wastewater treatment. In contrast, suburban wastewater treatment primarily relies on biological processes, with advanced oxidation and other sewage treatment techniques being less common, resulting in a lower capacity for treating antibiotics in wastewater. The discharge of antibiotics from WWTPs into natural water bodies can lead to a decrease in the self-purification capacity and pollution load of the receiving water bodies, affecting the ecological health of river water environments [15]. Although the mass concentration of antibiotics in surface water is generally between ng/L and mg/L, they are sufficient to have harmful effects on exposed ecosystems or organisms [16]. It is necessary to conduct an assessment of their discharge volume and ecological risks [17]. In order to better understand the temporal variation in antibiotics in WWTPs and their removal rate and potential ecological risks, this study investigated the following: (1) the temporal variation in four types of nine antibiotics in influent and effluent urban and suburban WWTPs; (2) estimating the usage and annual discharge of antibiotics in the urban and suburban environment based on per capita pollution load.

2. Materials and Methods

2.1. Experimental Reagents and Instruments

Ultra-high purity compounds (>99%) of nine antibiotics, including roxithromycin (macrolides), ofloxacin (quinolones), norfloxacin(quinolones), ciprofloxacin (quinolones), tetracycline (tetracyclines), chlortetracycline (tetracyclines), oxytetracycline (tetracyclines), sulfadiazine(sulfonamides) and sulfamethoxazole (sulfonamides), were bought from Sigma-Aldrich (St. Louis, MO, USA). The standard concentrations of each antibiotic were 1000 μg/mL, with a purity of >99% (Tianjin Alta Technology Co., Ltd., Tianjin, China). Acetonitrile, methanol, and formic acid were chromatographically pure (Thermo Fisher Corporation, MA, USA), and anhydrous sodium sulfate, sodium chloride, sodium dihydrogen phosphate dodecahydrate, disodium acetate tetraacetate, etc., were all chemically pure (Sinopharm Chemical Reagent Co., Ltd., Beijing, China). All solutions were prepared using Milli-Q water.

2.2. Sample Collection and Processing

Seasonal sampling campaigns were conducted in 2023 [January to March (winter), April to May (spring), July to August (summer), and October (autumn)] in Tangshan. Samples were collected from influent and effluent WWTPs in the urban ($n = 2$) and suburban areas ($n = 2$). The flow scheme of WWTPs is shown in Figure 1. To reduce experimental errors, instantaneous water samples were collected every 2 h for a total of 4 times within 1 day. The collected water samples were mixed evenly and stored in brown glass bottles to avoid light. They were transported back to the laboratory in an ice bath within 24 h. After filtration with a 0.45 μm glass fiber membrane, 500 mL was accurately measured, 0.25 g Na_2EDTA was added, and the pH was adjusted to about 3.0 with H_3PO_4. The samples were stored at 4 °C, and solid phase extraction was completed within 48 h.

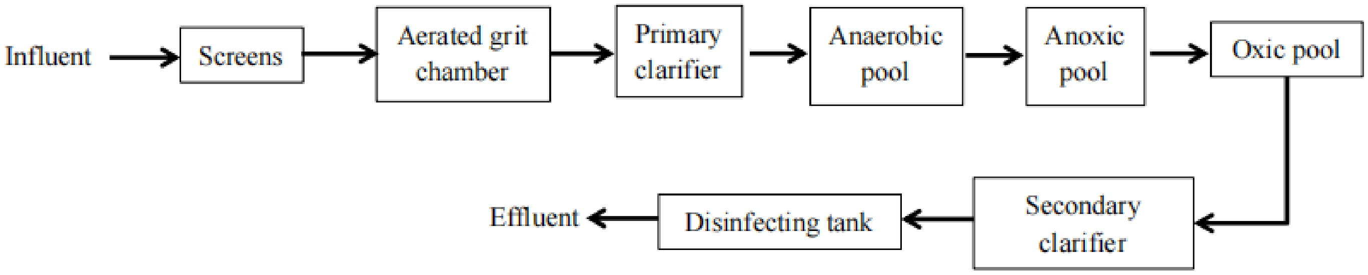

Figure 1. Schematic diagram of wastewater treatment plants (WWTPs) in Tangshan.

2.3. Qualitative and Quantitative Analysis

Take 1 L of the water sample and use a vacuum filtration device to pass it through a 0.45 μm filter membrane. Use a fully automated solid phase extraction instrument and an HLB extraction column to complete the preliminary extraction and concentration. First, activate the extraction column with 10 mL of methanol and 10 mL of ultrapure water in sequence; then extract 1 L of water sample at a rate of 10 mL/min; after completion, use high-purity nitrogen to dry the extraction column for 20 min; finally, wash the extraction column with 5 mL of dichloromethane, 5 mL of ethyl acetate, 5 mL of n-hexane, and 5 mL of methanol in sequence, repeating twice. After extraction, use a rotary evaporator to concentrate the eluent to about 1 mL and transfer it to a test tube; then, use a nitrogen-blowing instrument to blow it nearly dry and dilute it to 1 mL with methanol. The diluted sample passes through a 0.22 μm organic filter membrane and is transferred to a liquid phase vial for testing. The pretreated samples were analyzed using UPLC-MS/MS (QTRAPTM5500 LC/MS/MS system, SCIEX, MA, USA), employing a Waters Cortecs T3 column (2.1 mm × 100 mm, 2.7 μm). The injection volume for liquid chromatography was 2 μL, with a flow rate of 0.3 $\text{mL}\cdot\text{min}^{-1}$ and a column temperature of 40 °C. The mobile phase was a gradient elution of 0.1% formic acid aqueous solution and acetonitrile. The qualitative and quantitative analysis of antibiotics was carried out using the multiple reaction detection scanning mode (MRM) and electrospray ionization mass spectrometry (ESI/MS) positive and negative ion modes [18,19].

2.4. Quality Control

Using methanol as the solvent, the standard stock solution was diluted to 0.1, 2, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 μg/L. Linear regression was performed between the concentration of the antimicrobial drug and the corresponding peak area to draw a standard curve for the antibiotics. The standard curve had a good linear correlation within the corresponding linear range (correlation coefficient $R^2 > 0.99$).

2.5. Ecological Risk Assessment Method

The ecological risk assessment is a scientific evaluation of the potential damage of toxic and harmful pollutants to the ecological environment through quantitative characterization methods [20]. In this study, the risk quotient (RQ) was used to evaluate the ecological risk of antibiotics [20,21]. The calculation method of RQ is shown in Equation (1):

$$\text{RQ} = \text{MEC(PEC)}/\text{PNEC} \tag{1}$$

In the formula, MEC is the measured environmental concentration of antibiotics, PEC is the predicted concentration of antibiotics, and PNEC is the predicted no-effect concentration of antibiotics. In this study, the measured concentration of antibiotics, MEC, was used to calculate their risk quotient, and the predicted no-effect concentration (PNEC) was determined using the evaluation factor method. The chronic toxicity data (ChV) of antibiotics came from the Ecological Structure Activity Relationships Program (ECOSAR) predictive analyzer developed by the US Environmental Protection Agency. In this study, the ChV values for roxithromycin, tetracycline, chlortetracycline, oxytetracycline, ciprofloxacin, norfloxacin, ofloxacin, sulfadiazine, and sulfamethoxazole were 0.6, 20, 20, 20, 116, 114, 116, 0.101, and 0.068 mg/L, respectively. An extrapolation factor of 100 was selected to determine the PNEC of each antibiotic [22]. The PNEC values of each antibiotic are shown in Table 1. When $RQ < 0.1$, it is low risk; when $0.1 \leq RQ < 1$, it is medium risk; and when $RQ \geq 1$, it is high risk [23,24].

Table 1. PNEC (μg/L) of the target antibiotic in effluents from wastewater treatment plants (WWTPs).

Target Antibiotic	PNEC
Roxithromycin	1.5
Tetracycline	1.0
Aureomycin	1.0
Oxytetracycline	1.0
Ciprofloxacin	20,000
Norfloxacin	23,000
Ofloxacin	22,000
Sulfadiazine	15
Sulfamethoxazole	6.4

2.6. Estimation of Use and Emissions

The daily mass load of antibiotics per capita in the influent of WWTPs [μg/(d·person)] can reflect the use of antibiotics in the service area of WWTPs, as shown in Equation (2) [25]:

$$L_{\text{influent}} = (Q \times C_{\text{influent}})/P_{\text{total}} \tag{2}$$

In the formula, Q is the daily sewage flow of WWTP (m^3/day) (Table S1), C_{influent} is the average concentration of antibiotics detected in the influent of WWTP (ng/L), and P_{total} is the number of residents in the service area of WWTPs (Table S1). P_{total} in the urban and suburban areas of Tangshan were, respectively, provided by the Tangshan Municipal Design Institute. The usage amount of antibiotics (U, kg/year) and the mass load of antibiotics in the effluent of WWTP (M, g/year) are shown in Equations (3) and (4) [26–28]:

$$U = L_{\text{influent}} \times P_{\text{total}} \times 365 \times 10^{-9} \tag{3}$$

$$M = C_{\text{effluent}} \times Q \times 365 \times 10^{-6} \tag{4}$$

In the formula, L_{influent} represents the per capita pollution load of antibiotics [μg/(d·person)], and C_{effluent} represents the average detection concentration of target antibiotics in the effluent of WWTPs (ng/L).

3. Results and Discussion

3.1. Influent

The seasonal variation trend of the total antibiotics concentration in the inflow of WWTPs in the Tangshan area was the highest in winter, followed by spring, summer, and autumn (Figures 2 and 3). In spring, summer, and winter, the concentration of antibiotics in the inflow of urban WWTPs was higher than that of suburban WWTPs, while the opposite trend was observed in autumn (Figure 2). China announced that from 8 January 2023, COVID-19 infection will be adjusted from “Class A” to “Class B”. The monitoring data released by the National Influenza Center of China shows that since January 2023, the positive rate of influenza virus testing in the southern and northern provinces of China has continued to rise, and various regions have entered a high-incidence season of respiratory infectious diseases, with a significant increase in the number of infected individuals compared to previous years [29].

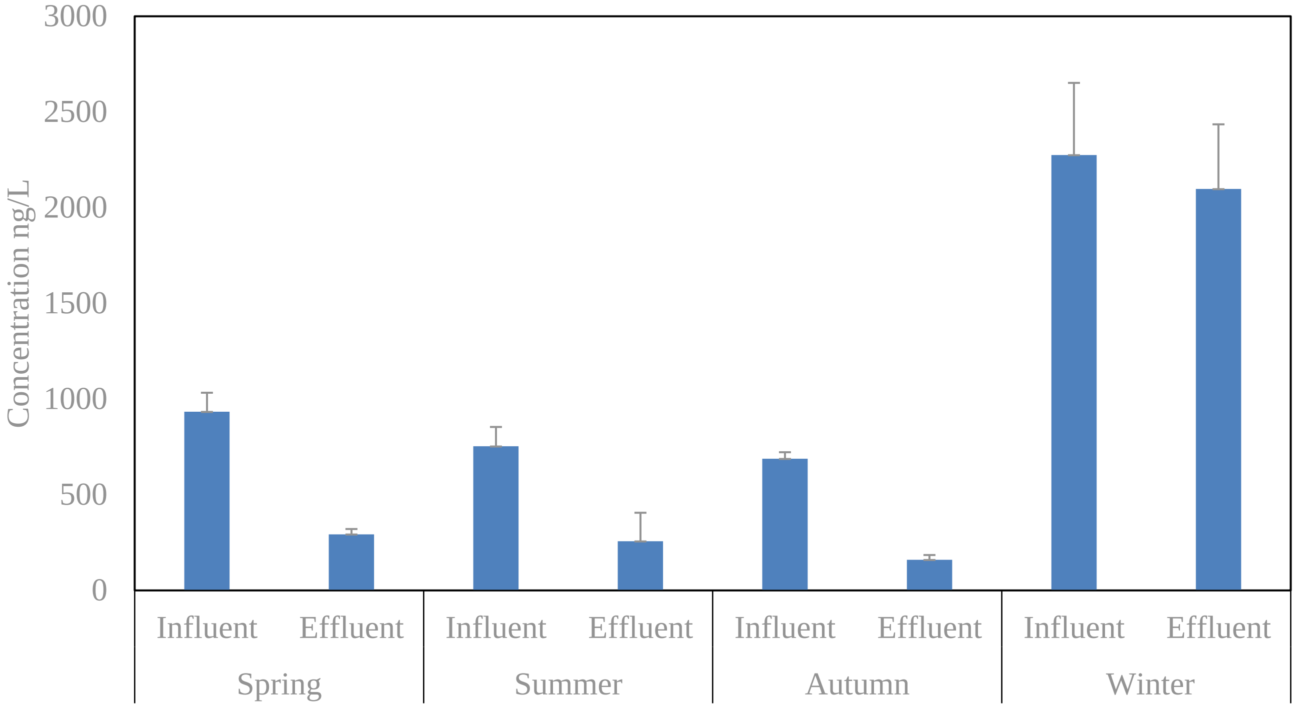

Figure 2. The occurrence of antibiotics in the influent and effluent of wastewater treatment plants (WWTPs) in Tangshan.

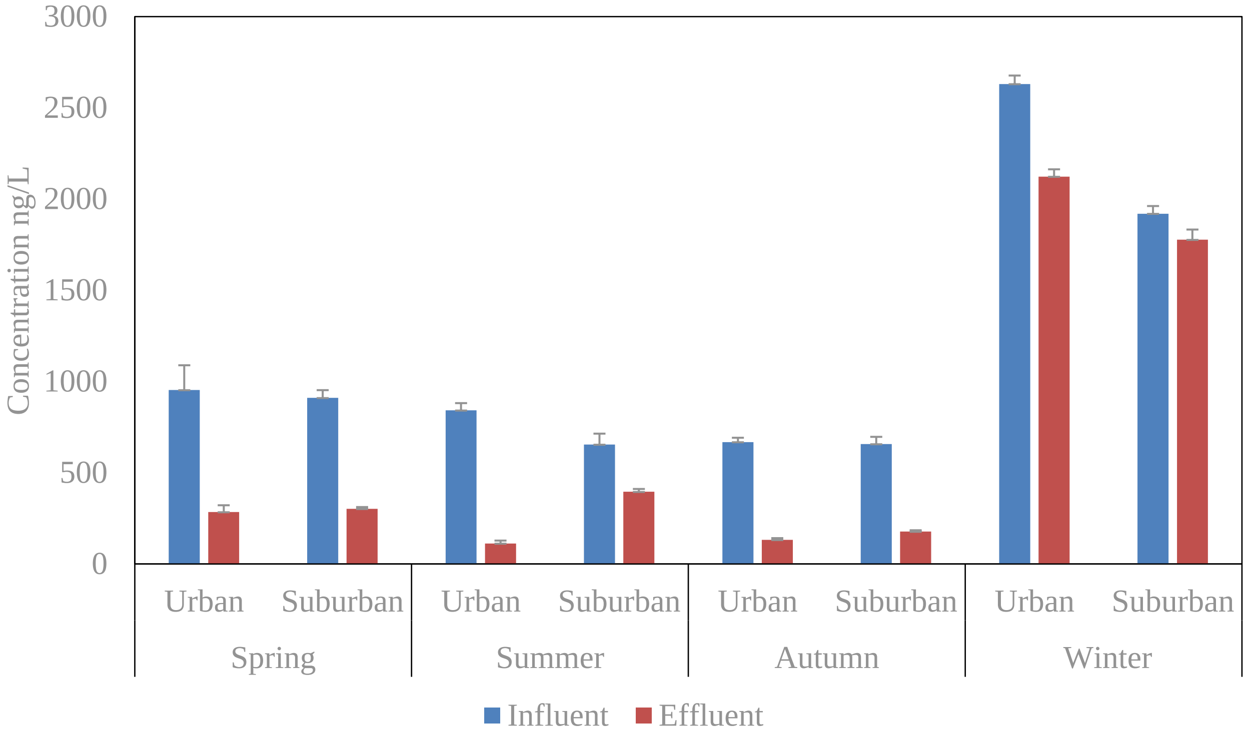

Figure 3. The occurrence of antibiotics in the influent and effluent of urban and suburban WWTPs in Tangshan.

This study selected the winter collection of inlet and outlet water samples from four WWTPs from January to March 2023. It is currently in a period of high incidence of respiratory diseases in the north, as represented by pneumonia. The increase in the use of antibiotic samples by urban populations has led to a much higher total concentration of antibiotics in the inlet water samples of WWTPs than in the other three seasons.

Among the quinolone antibiotics, ofloxacin had the highest concentration detected in the influent water of WWTPs in spring and autumn, followed by norfloxacin and ciprofloxacin. The concentrations of the three quinolone antibiotics detected in the influent water of urban WWTPs were higher than those in the suburban WWTPs (Table 2). In summer and winter, norfloxacin had the highest concentration detected in the influent water of WWTPs, while ofloxacin had the lowest concentration detected. Norfloxacin and ciprofloxacin had the highest concentrations detected in the influent water of urban WWTPs, while ofloxacin showed the opposite trend (Table 2). The concentrations of tetracycline antibiotics detected in the influent water of urban WWTPs were higher than those in the suburban WWTPs in all four seasons, except for oxytetracycline, which had lower concentrations detected in the influent water of urban WWTPs in spring and autumn compared to those in the suburban WWTPs. Among the three tetracycline antibiotics, tetracycline, chlortetracycline, oxytetracycline, and oxytetracycline had the highest concentrations detected in spring, summer, autumn, and winter, respectively, while chlortetracycline (suburban WWTPs), tetracycline (suburban WWTPs), tetracycline (suburban WWTPs), and chlortetracycline (suburban WWTPs) had the lowest concentrations detected (Table 2).

Table 2. The occurrence of antibiotics (ng/L) in the influent of wastewater treatment plants (WWTPs).

		Urban				Suburban			
Antibiotics		**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Roxithromycin	Mean	170.40	46.23	78.19	353.33	148.20	34.14	62.42	270.22
	SD	26.78	15.52	3.77	27.34	31.39	1.78	12.55	38.40
Tetracycline	Mean	76.83	18.03	14.72	104.23	61.19	14.17	12.88	71.22
	SD	9.66	4.29	3.89	43.13	12.34	1.75	2.49	17.70
Aureomycin	Mean	50.33	52.98	18.54	96.85	28.21	49.68	16.12	85.15
	SD	13.67	21.24	2.91	51.27	21.58	10.48	3.42	63.47
Oxytetracycline	Mean	40.41	44.87	36.22	256.70	42.30	36.62	62.33	101.67
	SD	15.46	1.56	6.86	17.96	1.41	7.79	33.01	10.80
Ciprofloxacin	Mean	99.83	123.96	79.68	451.79	86.73	107.31	68.25	316.84
	SD	15.32	22.69	12.52	19.76	18.37	31.82	13.77	9.56
Norfloxacin	Mean	122.10	135.17	97.50	458.49	105.76	116.56	84.89	321.42
	SD	17.91	17.68	15.32	20.05	22.41	24.70	18.04	9.87
Ofloxacin	Mean	125.37	47.87	122.62	491.53	106.53	87.72	89.93	422.56
	SD	64.02	1.18	21.57	14.12	53.25	2.19	4.76	93.96
Sulfadiazine	Mean	96.48	133.34	87.67	145.70	117.63	103.14	142.60	110.25
	SD	21.47	15.57	19.43	17.01	11.37	20.24	42.62	20.06
Sulfamethoxazole	Mean	171.60	235.80	140.55	275.04	213.65	121.98	164.26	217.34
	SD	36.39	27.53	49.70	32.12	25.01	16.23	53.80	46.54

During the study period, the macrolide antibiotic drug, roxithromycin, had the highest concentration detected in influent urban WWTPs, which was 1.15 times higher (spring), 1.35 times higher (summer), 1.25 times higher (autumn), and 1.31 times higher (winter) than that in suburban WWTPs (Table 2). The concentrations of sulfa antibiotics, sulfadiazine, and sulfamethoxazole, detected in influent suburban WWTPs were higher than that in urban WWTPs in spring and autumn while showing an opposite trend in summer and winter (Table 2). Sulfadiazine and sulfamethoxazole are antibiotics shared by humans and animals [30–32]. The breeding industry in suburban Tangshan is concentrated, and the use of veterinary antibiotics is high. Most veterinary antibiotics are excreted in the form of raw drugs or metabolites through animal feces and urine after administration and eventually enter the urban drainage system after sewage treatment [33–35]. Despite the legitimate reasons for their use, the current standards for the dosage of various veterinary antibiotics are inconsistent and imprecise, leading to the potential overuse of these drugs in livestock farming. This, in turn, raises the concentration of antibiotics in influent wastewater treatment plants within the farming region.

In influent WWTPs, (1) the concentration ranges of the nine antibiotics selected in this study, except for aureomycin, oxytetracycline, and ciprofloxacin, were much lower than those in Beijing (2018) in winter [36]; (2) the concentration of tetracycline antibiotics in summer was lower than existing research data, while the concentration of aureomycin and oxytetracycline in summer was higher than existing research data (except for Jiulongjiang River Basin), and the concentration of ciprofloxacin in quinolone antibiotics in summer and winter was higher than existing research data (except for Urumqi and Shihezi). In summer, the concentration of norfloxacin surpassed that of the Jiulongjiang River Basin yet remained lower than that of Urumqi and Shihezi at its peak. In summer, the concentration of ofloxacin was lower than that of the Jiulongjiang River Basin yet surpassed that of Yibin, Urumqi, and Shihezi [37–40]. (3) Among the sulfa antibiotics, sulfadiazine's concentration in summer and autumn surpassed that of the Jiulongjiang River Basin yet remained lower than that of Urumqi and Shihezi. The concentration of sulfamethoxazole in summer exceeded that of the Jiulongjiang River Basin, though its peak value was lower than that observed in Beijing (2019) [38–40] (Table S2).

3.2. *Effluent*

The removal effect of antibiotics in WWTPs in different seasons is closely related to treatment processes, operating parameters, influent properties, and types of antibiotics. Currently, most WWTPs employ biological treatment processes to degrade organic matter, including antibiotics. These processes primarily involve microorganisms in activated sludge attaching to the cell surface through adsorption and absorption. Different types of microorganisms utilize their metabolic capabilities to decompose and transform antibiotics. Ultimately, these microorganisms break down the molecular structure of the antibiotics into smaller organic compounds or CO_2 through enzyme production and oxidation, releasing corresponding metabolites. The seasonal variation characteristics of the detected concentration of antibiotics in the effluent of both urban and suburban WWTPs are shown in Figure 3. The total antibiotics concentration in effluent WWTPs in the winter was the highest, followed by spring, autumn, and summer. In winter, the concentration of antibiotics detected in the wastewater from urban WWTPs was higher than that from suburban WWTPs, while the trend was reversed in the other three seasons (Figure 3).

Currently, the WWTPs in Tangshan mainly use the A^2O (anaerobic–anoxic–aerobic) process for sewage treatment. The A^2O method is widely used in the sewage treatment system of northern China [41]. However, northern China experiences lengthy cold seasons, making it challenging for small-scale sewage biochemical treatment processes to operate stably [42]. Low temperatures decrease the activity of nitrifying and denitrifying bacteria, leading to a decline in the nitrogen removal efficiency of the A^2O process and challenges in its stable operation [43,44]. Previous studies have found that temperature has a significant impact on the nitrogen removal efficiency of the A^2O process. Nitrification reactions occur at 20–30 °C and almost stop at temperatures below 5 °C; denitrification reactions occur at 20–40 °C and rapidly decrease at temperatures below 15 °C. The winter temperatures in Tangshan and the surrounding areas are low, with average temperatures below 0 °C from January to February, which is not conducive to the degradation of antibiotics by microorganisms in activated sludge. Therefore, due to the impact of low temperatures on the efficiency of the A^2O process, the total concentration of antibiotics in the effluent of WWTPs in winter is one order of magnitude higher than that in spring, autumn, and summer (Figure 2).

In the effluent of both urban and suburban WWTPs, macrolide antibiotics, such as roxithromycin, have the highest detection concentration in spring, while this corresponds with quinolone antibiotics in autumn and winter (Table 3). In the effluent samples of suburban WWTPs in summer, the concentration of sulfa antibiotics in the effluent is higher than that in the influent (Table 3) [45]. Previous studies have also observed a similar phenomenon, where the concentration of sulfa antibiotics in the effluent after treatment by activated sludge processes has increased. This phenomenon may be due to the following reasons: (1) antibiotics adsorbed in activated sludge are released into the water, resulting in an increase in the concentration of these drugs in the effluent from WWTPs. (2) During the A^2O process, sulfa antibiotics are converted into other substances in the aerobic stage, and these substances are converted back into sulfa antibiotics in the anaerobic stage, resulting in an increase in the concentration of sulfa antibiotics in the effluent. In this study, the removal rates of nine antibiotics in urban and suburban WWTPs increased from 8.18% and 7.30% in winter to 70.14% and 66.82% in spring, and 79.58% and 73.91% in autumn. The removal rates of tetracyclines, quinolones, and sulfonamides in urban WWTPs were higher than those of macrolides in all four seasons, while the suburban WWTPs only followed the same trend in spring, autumn, and winter.

Table 3. Occurrence of antibiotics (ng/L) in the effluent of wastewater treatment plants (WWTPs).

		Urban				**Suburban**			
Antibiotics		**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Roxithromycin	Mean	107.23	22.82	35.68	322.58	104.93	15.80	24.75	248.95
	SD	3.17	9.78	6.62	15.06	13.54	2.64	1.91	38.02
Tetracycline	Mean	9.53	ND	1.85	93.60	7.51	2.98	ND	64.32
	SD	1.60	ND	2.62	39.69	4.22	0.87	ND	15.72
Aureomycin	Mean	5.63	6.13	ND	89.74	6.11	5.27	ND	78.82
	SD	0.95	0.71	ND	50.21	3.07	1.13	ND	60.22
Oxytetracycline	Mean	5.48	5.77	2.36	234.56	6.50	4.90	9.12	91.94
	SD	0.60	0.43	3.34	14.46	2.19	0.90	5.49	10.61
Ciprofloxacin	Mean	26.28	16.16	14.65	418.72	20.27	31.22	21.11	297.48
	SD	4.35	1.85	4.17	18.31	1.09	3.80	9.56	2.77
Norfloxacin	Mean	29.71	20.38	21.59	424.86	27.51	33.13	25.88	295.44
	SD	3.51	1.07	3.16	18.58	4.25	8.25	10.14	8.82
Ofloxacin	Mean	29.10	9.03	20.78	442.08	34.32	26.27	28.48	394.54
	SD	12.45	0.85	4.22	13.32	20.34	2.49	3.28	81.54
Sulfadiazine	Mean	34.22	12.23	139.90	135.16	42.88	133.59	28.73	101.28
	SD	7.38	0.75	1.70	15.78	5.16	14.88	12.17	17.56
Sulfamethoxazole	Mean	37.18	19.60	21.13	256.67	52.20	145.73	39.51	201.87
	SD	7.90	4.53	14.20	34.42	6.84	24.76	6.86	44.41

Note: ND: not detected.

In the effluent of WWTPs, (1) the nine antibiotics selected in this study (excluding tetracycline, aureomycin, oxytetracycline, and ciprofloxacin) exhibited lower winter concentration ranges in comparison to Beijing (2018) [36]; (2) the roxithromycin concentration in summer was lower than that in the Zijiang River Basin of central Hunan, while in spring and autumn, they were an order of magnitude higher than those in Shenyang [36,39,46,47]; (3) Quinolone and tetracycline concentrations in spring and autumn were an order of magnitude higher than those in Shenyang, while in summer, they were an order of magnitude lower than concentrations in Yibin; (4) Sulfa antibiotic concentrations in summer and autumn were higher than those reported by Beijing (2019) and Shenyang, and in summer, they were an order of magnitude higher than those in Yibin [36,46,47] (Table S3). The variation in the spatial and temporal distribution of antibiotic concentrations was significant. This variation is primarily attributed to a complex interplay of factors, including the treatment processes and surface temperatures employed by sewage treatment plants across different regions, the sources and composition of sewage within the service area, and the size of the population served by these WWTPs.

3.3. Ecological Risk Assessment

In the effluent of WWTPs in winter, the RQ values of roxithromycin, tetracycline, chlortetracycline, and oxytetracycline in urban areas were 0.22, 0.1 (0.09), 0.1 (0.08), and 0.23, while those corresponding to roxithromycin and oxytetracycline were 0.17 and 0.1 (0.09) in suburban areas. This indicates that these macrolides and tetracyclines are medium-risk antibiotics. In the other three seasons, the four categories of nine antibiotics had RQ values of ≤ 0.1 in the effluent of WWTPs and showed low-risk antibiotics. It is worth noting that macrolides, including roxithromycin, are medium-risk antibiotics in the effluent of urban and suburban WWTPs in winter, and there is a possibility of the overuse of these drugs by residents in Tangshan. Chen et al. used RQs to assess the ecological risks of antimicrobial drugs. The results showed that erythromycin, roxithromycin, tetracycline, chlortetracycline, sulfamethoxazole, and norfloxacin were high-risk pollutants in water bodies in China, accounting for 20.9% [48]. In winter, various respiratory diseases, including mycoplasma pneumoniae, influenza, adenovirus, and respiratory syncytial virus infections, are highly prevalent. The peak season for mycoplasma pneumoniae infection occurs from August to February of the subsequent year, with the highest incidence of around December to January of the following year [49]. Macrolide antibiotics, such as roxithromycin and clarithromycin, are stable to acidic and have a long half-life (35–48 h), a broad antibacterial spectrum, high bioavailability, are widely distributed in the body, and have significant efficacy, with minimal gastrointestinal irritation [50]. They have become the first choice for treating mycoplasma pneumoniae infection [51,52]. At present, the resistance rate of Mycoplasma pneumoniae to macrolides has been on the rise worldwide [53]. East Asia is the region with the most serious resistance to macrolide drugs for Mycoplasma pneumoniae in the world. Studies have shown that the resistance rate in some areas of China has reached over 90%.

3.4. Estimation of Usage and Sewage Discharge

The per capita pollution load, annual usage, and annual emissions of antibiotics in Tangshan are presented in Tables 4 and 5. The per capita pollution load of antibiotics was highest in spring, summer, and autumn for sulfamethoxazole, while the highest load in winter was for ofloxacin. From spring to winter, the per capita pollution load of antibiotics for urban residents was 9.63–13.74 times that of suburban residents, suggesting that urban residents may be at risk of antibiotic abuse (Table 4). In urban areas, the usage of roxithromycin (5.87 kg/a in spring), sulfamethoxazole (8.96 kg/a in summer), sulfamethoxazole (5.77 kg/a in autumn), and ofloxacin (17.76 kg/a in winter) significantly surpasses that of other antimicrobial agents. In contrast, the usage levels in suburban areas are as follows: sulfamethoxazole (0.17 kg/ain spring), norfloxacin (0.10 kg/ain summer), sulfamethoxazole (0.12 kg/ain autumn), and ofloxacin (0.32 kg/ain winter) (Table 4). It should be noted that the usage of antibiotics in urban areas was one order of magnitude higher than that in suburban areas (Table 5). After treatment by the A^2O process in the WWTPs, the four types of nine antibiotics selected in this study, including roxithromycin (3.87 kg/a), norfloxacin (0.73 kg/a), roxithromycin (1.39 kg/a), and ofloxacin (15.96 kg/a), were the highest in terms of emissions from urban WWTPs in spring, summer, autumn, and winter, respectively. Roxithromycin, sulfamethoxazole, sulfamethoxazole, and ofloxacin were the highest in terms of emissions from suburban WWTPs during the corresponding periods, respectively. The total usage of the nine antibiotics in urban and suburban WWTPs in 2023 was 32.55 and 0.75 kg/a, 30.11 and 0.58 kg/a, 24.29 and 0.57 kg/a, and 96.05 and 1.59 kg/a, respectively, while the total emissions were 9.86 and 0.25 kg/a, 3.83 and 0.33 kg/a, 4.84 and 0.15 kg/a, and 88.38 and 1.48 kg/a, respectively.

Table 4. Estimates of per capita pollution load of antibiotics [μg/(d·person)] in Tangshan.

	Urban				Suburban			
Antibiotics	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Roxithromycin	63.11	17.12	28.96	130.86	5.49	1.26	2.31	10.01
Tetracycline	28.46	6.68	5.45	38.60	2.27	0.52	0.48	2.64
Aureomycin	18.64	19.62	6.87	35.87	1.04	1.84	0.60	3.15
Oxytetracycline	14.96	16.62	13.41	95.07	1.57	1.36	2.31	3.77
Ciprofloxacin	36.98	45.91	29.51	167.30	3.21	3.97	2.53	11.73
Norfloxacin	45.19	50.06	36.11	169.81	3.92	4.32	3.14	11.90
Ofloxacin	46.43	17.73	45.41	182.05	3.95	3.25	3.26	15.65
Sulfadiazine	35.73	49.39	32.47	53.96	4.36	3.82	5.28	4.08
Sulfamethoxazole	63.56	87.33	52.06	101.87	7.91	4.52	6.08	8.05
Total	353.06	310.46	250.25	975.40	33.71	24.86	25.99	70.99

Table 5. Estimates of antibiotics use and emissions (kg/a) in Tangshan.

	Urban				Suburban			
	Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
Use								
Roxithromycin	5.87	1.49	2.90	12.54	0.13	0.03	0.06	0.23
Tetracycline	2.93	0.60	0.49	3.25	0.05	0.01	0.01	0.05
Aureomycin	2.01	1.66	0.64	4.20	0.03	0.04	0.01	0.09
Oxytetracycline	1.28	1.66	1.23	9.60	0.04	0.03	0.06	0.09
Ciprofloxacin	3.45	4.23	2.75	16.23	0.08	0.10	0.06	0.26
Norfloxacin	4.22	4.71	3.36	16.48	0.09	0.10	0.08	0.26
Ofloxacin	3.75	1.73	4.20	17.76	0.07	0.07	0.07	0.32
Sulfadiazine	3.24	5.07	2.95	5.54	0.09	0.09	0.10	0.10
Sulfamethoxazole	5.79	8.96	5.77	10.45	0.17	0.09	0.12	0.19
Emissions								
Roxithromycin	3.87	0.71	1.39	11.58	0.09	0.01	0.02	0.22
Tetracycline	0.33	0.00	0.03	2.90	0.01	0.00	0.00	0.05
Aureomycin	0.22	0.21	0.00	3.92	0.01	0.00	0.00	0.08
Oxytetracycline	0.19	0.20	0.04	8.75	0.01	0.00	0.01	0.08
Ciprofloxacin	0.90	0.57	0.48	15.05	0.02	0.03	0.02	0.24
Norfloxacin	1.04	0.73	0.75	15.27	0.02	0.03	0.02	0.24
Ofloxacin	0.90	0.32	0.70	15.96	0.02	0.02	0.02	0.30
Sulfadiazine	1.15	0.44	0.49	5.14	0.03	0.11	0.02	0.09
Sulfamethoxazole	1.26	0.66	0.95	9.81	0.04	0.11	0.03	0.18

4. Conclusions

Due to the high incidence of respiratory diseases, the use of antibiotics has increased, resulting in the highest concentration of antibiotics in the winter for both influent urban and suburban WWTPs. However, due to the low-temperature environment, the removal rate of antimicrobial drugs by the A^2O process in WWTPs is the lowest in winter. Based on the RQ method for evaluating the ecological risk of antibiotics, it was found that the RQ values of roxithromycin, tetracycline, aureomycin, and oxytetracycline in the winter effluent samples from urban WWTPs were 0.22, 0.1 (0.09), 0.1 (0.08), and 0.23, respectively, identifying them as medium-risk antibiotics. The RQ values of roxithromycin and oxytetracycline in the winter effluent samples from suburban WWTPs were 0.17 and 0.1 (0.09), respectively, identifying them as medium-risk antibiotics. In the other three seasons, the four categories of nine antibiotics selected in this study had RQ values ≤ 0.1 in the effluent of WWTPs, all of which were low-risk pollutants. In the study area of Tangshan, the per capita pollution load of antibiotics was highest in spring, summer, and autumn for sulfamethoxazole while the highest in winter for ofloxacin. The highest use and emissions of antibiotics in the urban in spring, summer, autumn, and winter were roxithromycin (5.87 and 3.87 kg/a), sulfamethoxazole (8.96 kg/a), and norfloxacin (0.73 kg/a), while the highest use and emissions in suburban were sulfamethoxazole (0.17 kg/a) and roxithromycin (0.09 kg/a), norfloxacin (0.10 kg/a) and sulfamethoxazole (0.11 kg/a), sulfamethoxazole (0.12 and 0.03 kg/a), and ofloxacin (0.32 and 0.30 kg/a) in the same seasons.

This study focuses solely on the temporal distribution of target antibiotics in influent and effluent WWTPs. Certain antibiotics are susceptible to hydrolysis and removal in aquatic environments. Research indicates that macrolide antibiotics are prone to hydrolysis. Tetracycline antibiotics are not stable in water; for instance, the hydrolysis rate of oxytetracycline increases with deviations from neutral pH (pH = 7) and rising temperatures, whereas sulfonamides and fluoroquinolones are resistant to hydrolysis. pH and temperature are significant factors influencing hydrolysis. Consequently, it is imperative to further investigate the impact of seasonal variations in pH and temperature at the end of the drainage systems and within the treatment units of WWTPs on the temporal distribution of antibiotics to elucidate the driving factors behind any temporal trends observed in these agents' distribution in the influent and effluent. Moreover, the adsorption of antibiotics by sewage plant sludge is a significant factor in enhancing the removal rate of these antibiotics. For antibiotics primarily removed through sludge adsorption (such as fluoroquinolones and sulfonamides), an extension in sludge retention time concurrently enhances their removal efficiency. However, the removal of certain antibiotics may not be impacted by sludge retention time. Hence, there is an urgent need for a comprehensive assessment of the physical adsorption and biodegradation of antibiotics in WWTPs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16111627/s1, Table S1: Waste Water Treatment Plant (WWTP); Table S2: Comparison of the concentrations of target antibiotics in effluents from WWTPs in other cities; Table S3: Comparison of the concentrations of target antibiotics in influents from WWTPs in other cities.

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