Monitoring of Amoxicillin and Cephalexin Antibiotics in Municipal WWTPs during Covid-19 Outbreak- A Case Study in Isfahan, Iran

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Abstract

Antibiotics are non-biodegradable drugs that used to inhibit the expansion and growth of microorganisms. The presence of antibiotics and their residues in the environments has resulted in an increased universal concern due to their contrary impacts on human healthiness and also the environment. Specially with the prevalence of Covid-19, the consumption of antibiotics to treat or prevent secondary infections have increased. Therefore, the presence of most prescribed antibiotics from β -lactam class including amoxicillin and cephalexin were studied at two municipal WWTPs in Isfahan. Analytical method was extracted and then detected via HPLC/UV. Samples were collected from 2 wastewater treatment plants (WWTPs) on 13 sampling periods over 2 months on February and March 2020 during the outbreak of Covid-19. In WWTP A, the average concentration of amoxicillin in influent, effluent, and its removal efficiency was equal to $509.64 \pm 161.97 \mu\text{g/l}$, $354.37 \pm 201.41 \mu\text{g/l}$, $33.69 \pm 30.22\%$, and the average concentration of cephalexin in influent, effluent, and its removal efficiency was $189.42 \pm 176.06 \mu\text{g/l}$, $44.35 \pm 43.72 \mu\text{g/l}$, $64.61 \pm 26.52\%$, respectively. In WWTP B, the average concentration of amoxicillin in influent, effluent, and its removal efficiency was equal to $2134.82 \pm 3031.53 \mu\text{g/l}$, $401.09 \pm 205.86 \mu\text{g/l}$, $54.82 \pm 33.29\%$, and the average concentration of cephalexin in influent, effluent, and its removal efficiency was $183.69 \pm 123.48 \mu\text{g/l}$, $40.64 \pm 33.02 \mu\text{g/l}$, $72.89 \pm 19.45\%$, respectively. According to statistical points of view, the concentration of antibiotics in both WWTPs had significant differences and no critical correlation between removal efficiency of antibiotics with other principle wastewater parameters was observed.

Introduction

In the past decade, the presence of emerging contaminants such as pharmaceutical compounds (PhCs) in the environment, especially aquatic environments, has increasingly received great attention (Tran et al. 2018). Effluents from wastewater treatment plants (WWTPs) are the primary and constant sources of PhCs to the aquatic environments (Shraim et al. 2017). The reason is that conventional treatment plants are usually designed to eliminate nitrogen, phosphorous, and biodegradable carbon (Mirzaei et al. 2018b, Shokoohi et al. 2017) and they cannot remove PhCs efficiently (Shokoohi et al. 2017).

One of the most important groups of PhCs, with high usage and worldwide consumption, is antibiotics (Rodriguez-Mozaz et al. 2020). Antibiotics are used to deal with infections in humans or animals (Ngigi et al. 2020) and based on their characteristics, there are different types of them (Bilal et al. 2020). On the report of the World Health Organization (WHO), the ability of antibiotics to develop antimicrobial resistance genes in pathogenic bacteria is a critical concern to different aspects of lives today (Rodriguez-Mozaz et al. 2020, Shokoohi et al. 2020). Specially at the beginning of 2020 an outbreak of a respiratory disease called COVID-19 rapidly progressing worldwide and affecting humans of all ages with the high number of deaths (Miranda et al. 2020). With the prevalence of Covid-19, the consumption of antimicrobial drugs to treat or prevent secondary infections have increased. Current overuse of antibiotics could accelerate the emergence of the next global public health crisis that caused by resistance of

different types of microorganisms against antibiotics (Chen et al. 2021, Miranda et al. 2020) and scientists are concerned about their side effects (Usman et al. 2020).

Based on published data, the rate of antibiotic prescribing in developing countries is much higher than in developed countries (Alighardashi et al. 2014). Results to the investigation of national and regional monitoring organizations from 71 countries along with analyzed data from scientific writings during the past decade has shown that the use of antibiotic is constantly increased globally (30%), mainly by rising demand in low- and middle-income countries (Rodriguez-Mozaz et al. 2020). Specially in Asia, which make up almost 60% of the world's population, antibiotics and other drugs are easily available on the markets (Tran et al. 2018). Iran, in terms of using drug, is between the top twenty countries in the world (Aali et al. 2020) and about 13% of the pharmaceutical market in this country is made up of antibiotics (Rezazadeh 2016) and among oral antibiotics, beta-lactams are along with the four most widely used antibiotics in this country (Zaboli et al. 2012). In total, the consumption of antibiotics in Iran reaches 16 times the world standard (Aali et al. 2020, Neisi et al. 2017).

The widespread use of antibiotics has resulted in an increased concern worldwide owing to their recurrent detection in different environmental media such as coastal (Dougherty et al. 2010), surface (Hanna et al. 2018, Ngigi et al. 2020, Shi et al. 2020), and ground (Barnes et al. 2008, Focazio et al. 2008) waters, soil (Hanna et al. 2018), edible part of vegetables (Patel et al. 2019) and etc. that have potential hazards for humans and environment (Ngigi et al. 2020).

About 10 – 90% of antibiotics depending on their chemical properties will be excreted out of the body unchanged or as metabolites through urine and body excretions (Shokoohi et al. 2017). For example over 80% of oral administration of amoxicillin, that is the most widely used antibiotic for the treatment of bacterial infections, would be excreted out of body after 2 hrs. of consumption (Chaba & Nomngongo 2019). The excreted antibiotics will be delivered into the wastewater (Honda et al. 2018, Shokoohi et al. 2017). Due to water scarcity, climate change, urbanization, and regional drought, the use of WWTPs effluent to irrigate agricultures in arid and semi-arid regions is usual (Pan & Chu 2017) and especially in Iran, Isfahan, which is in the semi-arid region, there is no exception. Therefore, having knowledge on the fate of antibiotics in WWTP influent and effluent and the removal efficiency of them in WWTPs is important and can estimate their potential impacts on ecology and human health (Kim & Aga 2007, Lacey et al. 2012).

The concentration of antibiotics in WWTPs effluents greatly depends upon several factors such as the design and operational conditions of WWTP (Kim & Aga 2007), treatment procedure, hydraulic retention time (HRT), and solid retention time (SRT) (Gao et al. 2012). Also since the usage patterns of antibiotics are different, the types and concentrations of them detected in WWTPs differ from country to country or even from plant to plant (Gao et al. 2012).

The monitoring of antibiotics in WWTPs have been the subject of numerous studies. Some studies have manifested that antibiotics are not eliminated thoroughly in common WWTPs (Chang et al. 2010, Karthikeyan & Meyer 2006, Mirzaei et al. 2018b, Shokoohi et al. 2017, Shraim et al. 2017, Verlicchi et al.

2013). Sara Rodriguez-Mozaz et al. in 2020 analyzed the effluent of 13 WWTPs in 7 European countries and detected 17 antibiotics in effluents (Rodriguez-Mozaz et al. 2020). Yungang Shi et al. in China analyzed 43 antibiotics in WWTP and among them, 23 antibiotics were found both in influent and effluent (Shi et al. 2020). Ngoc Han Tran et al. in a study have shown that the concentration of PhACs, specially antibiotics, in wastewater and effluent of Asian countries is more than European and North American countries (Tran et al. 2018). In Pakistan, Rabeea Zafar et al. detected 5 antibiotics in the effluents of two WWTPs entered into the river. The concentration of detected antibiotics was high and at the range of $\mu\text{g/L}$ (Zafar et al. 2020), much more than found in other counties effluents (ng/l or less). Reza Shokoohi et al. indicated the presence of amoxicillin, cefixime, and imipenem in the effluent of WWTPs at high concentrations around $1.6\text{--}10.7\ \mu\text{g/L}$ (Shokoohi et al. 2017). Researchers in Beijing, China investigated antibiotics in eight WWTPs and out of 22 antibiotics, 14 were detected. The maximum concentration of antibiotics was $3.1\ \mu\text{g/L}$ in influent and $1.2\ \mu\text{g/L}$ in effluents. Antibiotics were also found in all sludge samples at the concentrations around 1.0×10^{-1} to $2.1\times 10^4\ \mu\text{g/kg}$ (Gao et al. 2012). In some studies, the reported concentration of antibiotics in effluent is higher than influent (Gao et al. 2012, Jelic et al. 2012, Mirzaei et al. 2018b, Yan et al. 2014). Roya Mirzaei et al. found 7 antibiotics among of 9 antibiotics in both the influent and effluent of 2 WWTPs in Tehran, Iran and some of them detected in effluent but not detected in influent (Mirzaei et al. 2018b).

It should be noted that all studies have been conducted before the outbreak of Covid-19 in the world. Studies showed that the consumption of antibiotics has been increased after the outbreak of Covid-19 (Chen et al. 2021, Usman et al. 2020) and therefore, their concentration in WWTPs is expected to be increased. Nikaeen et al. in a study have shown an increase in the concentration of antibiotics in the WWTPs of Isfahan during the outbreak of COVID-19 (Gholipour et al. 2021). Amoxicillin and cephalixin are 2 antibiotics from β -Lactams that are among the most commonly used antimicrobial drugs in Iran and Isfahan (Benito-Peña et al. 2009, Verdier et al. 2011). In addition to the high prescription, they are also cheap and most of people in Iran buy and use them arbitrary. Specially the outbreak of COVID-19 along with flu during the winter can lead to overuse of these antibiotics. Therefore, the aim of this study was to monitor Amoxicillin and Cephalixin antibiotics in the influent and effluent of municipal WWTPs and evaluate the removal efficiency of them in winter 2019 during the outbreak of COVID-19.

Materials And Methods

Chemicals and reagents

The analytical standards of target antibiotics including amoxicillin and cephalixin were of high purity grade (N99%) and were purchased from Sigma-Aldrich (Steinham, Germany). Sodium phosphate salt also was purchased from Sigma-Aldrich (Steinham, Germany). The HPLC grade acetonitrile (ACN) and acetic acid (85%) were purchased from Merck (Darmstadt, Germany). CA Syringe Filters were purchased from Membrane Solutions Company (U.S.A). Mili-Q water was purchased from SKY Company (Iran).

Target antibiotics

More than 65% of the antibiotics that are been used in the world are β -lactam antibiotics. Also in Iran, 32.6% of used antibiotics belong to β -lactams (penicillin, amoxicillin, and ampicillin), of which amoxicillin is the most widely used (AR et al. 2016). According to a study conducted in 2012, it was found that among oral antibiotics, β -lactams are among the four groups of the most widely used oral antibiotics in Iran (Rezazadeh 2016). Therefore, the list of all antibiotics distributed to the clinics and public hospitals in Isfahan for the year 2020 was obtained from the Directorate General of Health Affairs of Isfahan. Two of the most commonly administered antibiotics from β -lactams, including amoxicillin and cephalexin, based on their amounts of used and so the probability of being detected in wastewater, have been chosen to be inquired in this study.

Sampling sites

This study was performed on February and March 2020, at the prevalence of Covid-19 in the world and also in Iran, at two municipal WWTPs in Isfahan, Iran. WWTP A has been designated in 2 modules, and each of them discharges a population of approximately 250,000 inhabitants (500,000 residents in total) and treats 100,000 m³/day of municipal wastewater per day. The treatment procedure is including anaerobic ponds, aeration lagoons, and facultative lagoons. The effluent of facultative lagoons enters the transmission channel from the outlet and reuse. Figure 1 shows the flow diagram of treatment process in WWTP A.

WWTP B has been designed in 2 modules, and discharges a population of nearly 900,000 inhabitants and treats 130,000 m³/day of municipal wastewater. The treatment procedure is conventional activated sludge. The effluent of the secondary clarifier, after chlorination, is discharged into the river. Figure 2 shows the flow diagram of treatment process in WWTP B.

Sample collection procedure

In this study, during 2 months from each WWTP, a total of 13 combined samples from the influent and effluent were taken according to standard method, and because two treatment plants were studied, the total number of samples from the two treatment plants were 52 samples.

For sample collection, all used glass were soaked in 10% nitric acid, washed with water, and finally with distilled water before use (Shraim et al. 2017) and let them to dry.

Influent samples were collected after screening at both studied WWTPs, and effluent samples were obtained from the transmission channel of facultative lagoons in WWTP A, and the effluent of secondary clarifier in WWTP B. Samples were collected in 1L silanized dark glass bottles and rinsed twice with the influent or effluent before sampling. The collected samples were stored at 4°C in a cool box on the way to the laboratory (Zafar et al. 2020) and being investigated as soon as entering the lab.

Since the pH of the samples was close to neutral values (pH 7.2–7.6), so there was no need of pH adjustment.

Preparation of standard solutions and samples

Each of standard solution of target antibiotic was prepared freshly in a concentration of 1 mg/mL and dissolved in Mili-Q water as follows: approx. 0.025gr of each antibiotic was strictly weighed and dissolved in 25 mL Mili-Q water. In order to reduce the degradation of antibiotics, the test tubes containing the stock solutions were covered with aluminum foil and stored at 4°C in refrigerator (Zafar et al. 2020).

All samples were centrifuged at 4,000 rpm for 5 minutes. Then the transparent part was separated and passed through the CA Syringe Filters. Then, 1ml of the filtered samples was injected into HPLC-UV.

2.6. Analytical method

The concentration of target antibiotics including amoxicillin and cephalixin in the samples was determined by an HPLC system (Jasco PU-2080, Tokyo, Japan) which was equipped with a quaternary mixing pump, an inline vacuum degasser, automatic injector (AS-2055 Plus), a C18 column (150 × 4.6 mm, Germany), packed with 5 µm particles for the separation, and an UVVis detector (UV-2075 plus).

The mobile phase that was used containing a gradient of Mili-Q water, acetonitrile, NaH₂PO₄ 1M, and acetic acid 1N (909:80:10:1v/v).

Multiple runs were performed with different conditions including flow rate, run time, and wavelength to analyze each target antibiotic. The HPLC method for each analytes is presented in Table 1.

Table.1. Optimal HPLC conditions for the analysis of target antibiotics, their related calibration curves, and detection limits

Antibiotics	Wavelength (nm)	Retention Time (min)	Calibration Curves		LOD (µg/L)	LOQ (µg/L)	Recovery (%)
			Equation	R ²			
Amoxicilin	237	4/475	y = 1419.7x -79037	0.9905	31.3	103.6	91.3
Cephalexin	274	6/908	y = 1667.2x + 5140.6	0.9996	6	98.3	93.5

Method validation

Method validation was based on the linearity response of the results regression coefficient or R^2 which is calculated using Excel software. Based on available sources, a regression coefficient above 0.99 is the best condition to ensure the linearity of the results and as mentioned in Table 1, for our target antibiotics R^2 was above 0.99 which indicates appropriate accuracy.

The next indicator to ensure the accuracy of the measurements is to calculate the LOD (limit of detection) and LOQ (limit of quantification), and all obtained results should be higher than them. LOD and LOQ would be calculated according to Eqs. 1 and 2 as follow:

$$LOD = 3.3 \left(\frac{S_y}{S} \right)$$

1

$$LOQ = 10 \left(\frac{S_y}{S} \right)$$

2

In Eqs. 1 and 2, S_y is standard deviation of the response, and S refers to slope of the calibration curve. At the end, according to Eq. 3 the total method recovery will be calculated.

$$Recovery(\%) = \frac{RealConcentration}{ArtificialConcentration} \times 100$$

3

The results obtained from the validity of the results are summarized in Table 1. The chromatogram diagrams of target antibiotics obtained from HPLC method for targeted antibiotics can be seen in Fig. 3.

Calculation of removal efficiency in WWTPs

In present study, the concentration of target antibiotics in influent and effluent of WWTPs obtained and then according to the results removal efficiency of antibiotics were calculated. Removal efficiency of target antibiotic in aqueous phase was calculated using Eq. 4 (Zhou et al. 2013) :

$$RemovalEfficiency(\%) = \frac{C_0 - C}{C_0} \times 100$$

4

In Eq. 4, C_0 refers to the concentration of target antibiotic in influent and C is the concentration of target antibiotic in effluent.

Statistical analysis

Data analysis was performed using SPSS software version 20, and average, mean, minimum, maximum, and standard deviation were calculated.

Then, in order to check the normality of the data, Kolmogorov-Smirnov Test was performed. To investigate significance dissimilarities among the concentrations of antibiotics in influent at both WWTPs Mann-Whitney test was used. Also the correlation of data was examined by Spearman method.

Results And Discussion

Concentrations of target antibiotics in WWTPs

Target antibiotics in real samples were quantified in the aqueous phase of the two WWTPs with a detection frequency of 100%. The concentrations of target antibiotics including amoxicillin and cephalixin in WWTPs A and B during the study period are presented in Fig.4.

In WWTP A, the average influent concentration of amoxicillin and cephalixin were 509.64 ± 161.97 and 189.42 ± 176.06 $\mu\text{g/L}$, respectively. Also, the average effluent concentration of amoxicillin and cephalixin were 354.37 ± 201.41 and 44.35 ± 43.72 $\mu\text{g/L}$, respectively. In WWTP B, the average influent concentrations of target antibiotics were 2134.82 ± 3031.53 (amoxicillin) and 183.69 ± 123.48 $\mu\text{g/L}$ (cephalexin), and the average concentration of amoxicillin in the effluent was 401.09 ± 205.86 $\mu\text{g/L}$ and cephalixin 40.64 ± 33.02 $\mu\text{g/L}$. As can be seen in Fig.4-A1 in WWTP A, the maximum influent concentration of amoxicillin was 718.44 $\mu\text{g/L}$ and the minimum influent concentration was 138.99 $\mu\text{g/L}$ and the maximum effluent concentration of amoxicillin was 674.6 $\mu\text{g/L}$ and the minimum effluent concentration was 31.3 $\mu\text{g/L}$. For cephalixin in WWTP A, according to Fig.4-A2 the maximum influent concentration was 655.21 $\mu\text{g/L}$ and the minimum influent concentration was 29.7 $\mu\text{g/L}$ and the maximum and minimum effluent concentration was 184.48 and 6 $\mu\text{g/L}$, respectively. In WWTP B, as could be seen in Fig.4-B1, the maximum influent concentration of amoxicillin was

9032.41 $\mu\text{g/L}$ and the minimum influent concentration was 501.77 $\mu\text{g/L}$ and the effluent concentration of amoxicillin was 801.17 (maximum) and 122.47 $\mu\text{g/L}$ (minimum), respectively. Fig.4-B2 shows the maximum concentration of cephalixin 425.43 $\mu\text{g/L}$ (influent) and 116.4 $\mu\text{g/L}$ (effluent) and the minimum concentration of cephalixin 78.61 $\mu\text{g/L}$ (influent) and 6.34 $\mu\text{g/L}$ (effluent), respectively. As can be seen in Fig.4-C1 and C2, in both WWTPs amoxicillin had the highest and cephalixin had the lowest concentration in the influent of both WWTPs. In WWTP A, the median influent concentration of amoxicillin and cephalixin was 558.66 and 118.9 $\mu\text{g/L}$, and the median influent concentration in WWTP B was 862.2

µg/L (amoxicillin) and 136.5 µg/L (cephalexin), respectively. The results show that amoxicillin has the higher consumption than cephalexin in Isfahan.

Amoxicillin is the most widely used antibiotic and over 80% of oral administration of it would be excreted out of body after 2 hrs. of consumption, and reported shows its presence in surface water, domestic and industrial wastewater, and hospital wastewater in concentration ranges of ng/L to mg/L (Chaba & Nomngongo 2019) and our findings were at the concentration of µg/L. In other studies, Ngoc Han Tran et al. reported the average concentrations of amoxicillin in influent 6516 ng/l and effluent 1670 ng/l of Asian countries, and 190 ng/l in effluent of European countries (Tran et al. 2018). In comparison with, the concentration of this antibiotic in influent and effluent of Isfahan WWTPs is too high. On the other study, Sara Rodriguez-Mozaz et al. analyzed the concentrations of antibiotics in the effluents of 13 municipal WWTPs in European countries and cephalexin was detected at concentrations ranging from 37-308 ng/L in 10 sample sites (Rodriguez-Mozaz et al. 2020). Amjad Shraim et al. reported the average concentration of cephalexin in influent and effluent 1.88 ng/ml and 1.55 ng/ml, respectively (Shraim et al. 2017). According to the results, it is observed that the average concentration of target antibiotics in influent and effluent of municipal WWTPs in Isfahan is much higher than other studies and this indicates the high consumption of these antibiotics in Iran, and Isfahan. It should be noted that other studies were conducted before the prevalence of COVID-19 viruses. So the main reason for the high concentration of antibiotics in aimed WWTPs is that antibiotics measured at the beginning of the prevalence of COVID-19 and the consumption of amoxicillin and cephalexin antibiotics have been increased at that time. Increased in using of antibiotics during the outbreak of COVID-19 has been reported in scientific articles (Chen et al. 2021, Miranda et al. 2020). Specially in Isfahan, that our studied plants are located, Nikaeen et al. reported an increase in the concentration of antibiotics in the wastewater of Isfahan city during the outbreak of COVID-19. It is mentioned that based on the distribution of patients and hospitals in Isfahan, the highest inflow of wastewater of COVID-19 patients had been to the WWTP B, and WWTP A was at the second rank (Gholipour et al. 2021). Therefore, it is expected that the concentration of antibiotics in WWTP B should be higher, which is completely true and the concentration of target antibiotics in WWTP B is higher than WWTP A, and the results obtained is consistent with this research.

Removal percentage of target antibiotics in two WWTPs

Municipal wastewater treatment plants are not able to completely remove pharmaceutical compounds, specially antibiotics, and the removal efficiency in each municipal wastewater treatment systems is different. In most previous studies, the removal efficiency of antibiotics in municipal WWTPs is less than 80%, and rarely removal efficiency of 100% has been observed (Shokoohi et al. 2017).

The removal percentage of target antibiotics in WWTPs A and B are shown in Fig.5. As it illustrated in Fig.5, in both WWTPs the removal efficiency of cephalexin is higher than amoxicillin. In WWTP A, the average removal efficiency of cephalexin is 64.61% and amoxicillin is 33.69% in this treatment plant. In WWTP B the average removal efficiency of cephalexin is 72.89% and amoxicillin 54.82%. In general, it seems that WWTP B was better in removed target antibiotics. In some previous studies activated sludge

is mentioned as a useful and available technology to eradicate pharmaceuticals from wastewater systems (Park & Oh 2020, Pilli et al. 2020, Quintelas et al. 2020, Wang et al. 2020) and our findings show that WWTP B by activated sludge system have removed antibiotics better than WWTP A.

In other studies Yungang Shi et al. in China estimated the average removal efficiency of antibiotics in municipal WWTPs at 57-95% (Shi et al. 2020). Despite the high concentration of antibiotics in effluent in this study, the removal efficiencies of target antibiotics are in this range, and it seems that two studied WWTPs have performed well at removing antibiotics. Reza Shokoohi et al. estimated the removal efficiency of amoxicillin in municipal wastewater treatment plant of Hamedan 55.66% (Shokoohi et al. 2017). Comparing the removal efficiency of amoxicillin in this study with the results obtained, the efficiency of WWTP B in removing amoxicillin was consistent with the results in Hamadan, but in general it seems that both our studied WWTPs have performed poorly in removing amoxicillin. Roya Mirzaei et al. reported the average removal efficiency of amoxicillin in the Ekbatan WWTP 75.21%, and 65.46% in other WWTP in Tehran (Mirzaei et al. 2018b). In previous studies, the efficiency of conventional activated sludge process at amoxicillin removal was predicted 84% and removal efficiency of cephalexin 76% (Mirzaei et al. 2018a). Therefore, it seems that WWTP B has removed cephalexin relatively well by activated sludge process, but it has performed poorly at removing amoxicillin. Amjad Shraim et al. in Saudi Arabia estimated the removal efficiency of cephalexin in the Almadinah Almunawarah municipal WWTP 18.16% (Shraim et al. 2017), comparing this number with the removal efficiencies obtained in this study, cephalexin removal efficiency in both treatment plants is more effective. The reason is that cephalexin is a biodegradable antibiotic and in Tehran WWTP with activated sludge process the removal efficiency has been reported for this antibiotic was up to 100% (Mirzaei et al. 2018b).

Statistical analyses antibiotic concentrations in WWTPs

In order to check the normality of the data Kolmogorov-Smirnov Test was performed. It should be noted that according to the P-Value ($P_{value} < 0.05$) in both treatment plants most of the data do not follow the normality distribution. Therefore, the nonparametric tests were applied for statistical analysis. Therefore, Mann-Whitney test was performed and the concentration of two target antibiotics compared at both WWTPs. According to the results of Mann-Whitney test, which are given in Table 2, it is clear that there is no significant difference between the concentration of cephalexin in WWTP A and B. But the concentration of amoxicillin in two WWTPs has significant difference. The reason is that wastewater from hospitals of Isfahan is discharged to WWTP B and also amoxicillin is among most commonly used oral antibiotics that are easily available on markets.

Table.2. Independent-Samples Mann-Whitney U Test results for antibiotics in WWTPs A and B

Parameters	Amoxicillin	Cephalexin
Total N	26	26
Mann-Whitney U	138.000	93.000
Exact Sig.(2-sided test)	0.000	0.687

To Investigating the correlation between target antibiotics and other main parameters of wastewater including Q, BOD₅, COD, and TSS which may affect the removal efficiency of antibiotics as well as their concentration in two studied WWTPs, Spearman test was performed and results are presented in Table 3. It should be noted that wastewater main parameters (Q, BOD₅, COD, TSS) were obtained from the laboratory of WWTPs. According to the results presented in Table 3, it is observed that in WWTP A there is not significantly correlation between the concentration of antibiotics in influent with other parameters, but in WWTP B there is more correlation. Specially there was a significant correlation between the Q_{in} of WWTP B and the concentration of amoxicillin at 99% confidence level in this WWTP. That is by increasing Q in WWTP B, the concentration of amoxicillin would be increased, too.

Table.3. Correlations Spearman among wastewater parameters in influent

Parameters	WWTP A		WWTP B	
	Amoxicillin	Cephalexin	Amoxicillin	Cephalexin
Amoxicillin_{in}	1.000	0.209	1.000	0.058
Cephalexin_{in}	0.209	1.000	0.058	1.000
Q_{in}	-0.304	-0.119	0.728**	0.265
BOD_{5 in}	0.275	0.007	0.270	0.645*
COD_{in}	0.275	0.007	0.621*	0.275
TSS_{in}	0.617*	-0.087	-0.198	-0.179

****.** Correlation is significant at the 0.01 level

***.** Correlation is significant at the 0.05 level

Spearman correlation test was also performed between the removal efficiency of target antibiotics and the removal efficiency of other main parameters (BOD₅, COD, TSS) in WWTPs A and B. According to the results that is presented in Table 4, in WWTP A there was no correlation between the removal efficiency of antibiotics and removal efficiency of other wastewater parameters. In WWTP B no correlation was observed between the removal efficiency of antibiotics and the removal efficiency of none of parameters and there was only a significant correlation between the removal efficiency of cephalixin and the removal efficiency of COD at 99% confidence level.

The reason for the different correlations in two studied WWTPs maybe is due to different treatment process of two studied WWTPs.

Table.4. Correlations Spearman among removal efficiencies in WWYPs

Parameters	WWTP A		WWTP B	
	R_Amoxicillin	R_Cephalexin	R_Amoxicillin	R_Cephalexin
R_Amoxicillin	1.000	-0.511	1.000	0.041
R_Cephalexin	-0.511	1.000	0.041	1.000
R_BOD ₅	0.215	-0.141	0.047	-0.006
R_COD	0.255	-0.174	-0.028	0.713**
R_TSS	-0.174	-0.315	0.423	0.283

****.** Correlation is significant at the 0.01 level

Conclusion

The concentrations of antibiotics were monitored during the outbreak of Covid-19 in 2 WWTPs of Isfahan and it is observed that the average concentration of target antibiotics including amoxicillin and cephalixin in influent and effluent of Isfahan WWTPs is much higher than other countries, and this indicates the careless use of antibiotics in Iran and Isfahan. In most previous studies in other countries, especially developed countries, the concentration of antibiotics in WWTPs is at the range of ng/L or even less. However, in the present study, the concentration of antibiotics is at the range of µg/L and this concentration is too high. One of the reasons is due to the occurrence of COVID-19 that causes the increase in use of antibiotics. But except clinical usages, unfortunately antibiotics are easily available on Iranian markets. Specially amoxicillin that is well known among people and they used it for flu, toothache, and any other infections arbitrary. The removal efficiency of antibiotics in studied wastewater treatment plants was not 100% and removal efficiency of amoxicillin was too deficient. Since the effluent of municipal WWTPs is generally reused, the presence of this concentration of antibiotics in the effluent is very important and there should be more attention. Specially due to water scarcity in Isfahan, farmers use effluents of WWTPs to irrigate agricultures and as mentioned before, antibiotics can accumulate in edible

parts of them and eating them by people or even animals would lead to antibiotic resistance in the future and treatable diseases will be untreatable and caused a lot of deaths. According to statistical point of view, the removal efficiency of the target antibiotics in two studied treatment plants was completely independent and no significant correlation was observed between the removal efficiency of antibiotics and the removal efficiency of other wastewater parameters such as BOD₅, COD, and TSS.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data analyzed during this study are included in this published article, and the datasets used during the current study (Q, BOD₅, COD, and TSS) are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Hossein Movahedian Attar: Supervision, Conceptualization, Review & Editing.

Mehri Samandari: Investigation, Writing Original Draft, Formal analysis, Laboratory experiments.

Karim Ebrahimpour: Methodology, Experimental advisor.

Farzaneh Mohammadi: Data investigation, Statistical analysis, Review & Editing.

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Figures

Figure 1

Flow diagram of treatment process in WWTP A

Figure 2

Flow diagram of treatment process in WWTP B

Figure 3

The chromatogram diagrams of target antibiotics

Figure 4

A-1: Concentration of amoxicillin in WWTP A, A-2: Concentration of cephalalexin in WWTP A, B-1: Concentration of amoxicillin in WWTP B, B-2: Concentration of cephalalexin in WWTP B, C-1: Box plot diagram of concentration of target antibiotics in WWTP A, C-2: Box plot diagram of concentration of target antibiotics in WWTP B

Figure 5

Removal efficiency of target antibiotics in WWTPs A and B

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