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Original Article

Measurement of ampicillin and penicillin G antibiotics in wastewater treatment plants during the COVID-19 pandemic: A case study in Isfahan

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Abstract

Background: In recent years, the world has faced with the COVID-19 pandemic, followed by a significant increase in the use of antibiotics to control the COVID-19 and other secondary infections. The non-biodegradable characteristics of antibiotics and their residues in the environment leads to increased microbial and drug resistance. Therefore, due to the high importance of antibiotics, two antibiotics, ampicillin and penicillin G, were studied in Isfahan municipal wastewater treatment plants (WWTPs).

Methods: Sampling was performed for two months during 13 sampling periods and antibiotics were measured using high-performance liquid chromatography with UV detector (HPLC/UV) instrument.

Results: Ampicillin and penicillin G were identified in all samples taken from the both WWTPs. The average concentration of penicillin G in WWTP E and S at the influent, effluent and its removal efficiency were 1050.54 ± 761.43 µg/L, 52.89 ± 49.27 µg/L, 89.80 ± 19.42%, 2055.12 ± 1788.08 µg/L, 143.01 ± 162.59 µg/L and 82.76 ± 21.85%, respectively. Also, the average concentration of ampicillin in WWTP E and S in the influent (796.44 ± 809.6 and 447.1 ± 322.39 µg/L), effluent (48.94 ± 24.25 and 90.31 ± 75.91 µg/L), and its removal efficiency (86.22 ± 19.84% and 66.85 ± 24.88%) were determined.

Conclusion: In two studied WWTPs, the concentration of antibiotics was higher during the COVID-19 pandemic in comparison with previous studies. The statistical analysis showed that there was no significant relationship between the concentration of antibiotics in WWTPs ($P < 0.05$). Also, the statistical results indicated that the correlation is not significant between removal efficiency of antibiotics and removal efficiency of wastewater main parameters.

Keywords: Anti-bacterial agents, COVID-19, Wastewater, High-pressure liquid chromatography, Ampicillin, Penicillin G

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Introduction

In December 2019, a respiratory disease called COVID-19 was reported in China and by the beginning of 2020 rapidly progressed all over the world, affected humans lives, and caused high number of deaths (1). Due to the COVID-19 pandemic, using of disinfectants and a variety of drugs for prevention and control strange viruses has significantly increased (2). A group of pharmaceutical compounds that are widely used all over the world is antibiotics (3), and especially due to the high prevalence rate of COVID-19, the consumption of them for the treatment and prevention of secondary diseases has been increased (1,2) and scientists are concerned about their side effects (4).

Antibiotics are antimicrobial drugs that treat infectious diseases in humans or animals (5) and since they have different chemical characteristics, there are of different types (6). The overuse of antibiotics has resulted in different issues and microbial resistance is one of them, which has caused concern and problems worldwide (1,2). Analyzing data from scientific documents, and also assessment of the results of national and regional monitoring organizations in 71 countries during the past decade, have shown an increase in the use of antibiotics (30%) (3). About 60% of the world's population are living in Asia, and unfortunately, you can buy antibiotics easily on its markets (7) and antibiotics make up about 13% of Iran's pharmaceutical market (8). Beta-lactam antibiotics are between the four most widely used antibiotics in Iran (9) and the total consumption of them in this country is 16 times more than the world standard (10,11).

Frequent detection of antibiotics in different environmental media such as coastal (12), surface (5,13,14), and ground (15,16) waters, soil (14), edible part of vegetables (17) etc due to extensive use of them would have potential risks for humans and environment (5). Antibiotics residue in various aquatic to terrestrial ecosystems can have adverse effects on a variety of microorganisms (5,13), and microbial activity can be inhibited by antimicrobials (5) and would have adverse effects on ecosystems (14). In addition, antibiotics residues have caused the increase of antibiotic-resistant genes and antibiotic-resistant bacteria (5), and it could make it difficult to control plenty of bacterial pathogens (13,18).

According to studies, most applied antibiotics would be excreted unchanged or in the form of their metabolites from the body (19) and delivered into the wastewater (19,20). Most existing wastewater treatment plants (WWTPs) are implemented for removing macropollutants (organic carbon, nitrogen and phosphorus) (19,21) and they cannot eliminate antibiotics completely (19). Many factors such as treatment process, operating conditions (22), solid retention time, and hydraulic retention time could affect the antibiotics concentration in WWTPs effluent. Due to the fact that the pattern of antibiotic usage in communities is different, their concentration and type identified in different environments would be also different (23).

Owing to critical issues such as drought, urbanization, climate change, and finally, the water scarcity, the use of effluent from WWTPs for irrigating agricultures is routine (24) and in Isfahan (Iran), which is located in the arid and semi-arid region, the use of effluents for agriculture purposes is essential. Therefore, if we have enough information about the concentration of antibiotics in the influent and effluent of WWTPs, as well as their fate, we can make accurate predictions of their possible effects on ecology and human health (22,25).

The concentration and fate of micropollutants such as antibiotics have been monitored in a large number of studies and the results show that antibiotics could not be simply removed using conventional treatment processes (19,21,26-29). In a study conducted in China, the concentration of 43 antibiotics was measured in the influent and effluent of WWTPs, 23 antibiotics were identified in both influent and effluent samples (13). Another study in Asian countries on treatment plants found that the concentration of antibiotics in these countries is much higher than North American and European countries (7). Rodriguez-Mozaz et al in a study in 2020 in 7 European countries, analyzed antibiotics in 13 treatment plant effluents and identified 17 types of antibiotics in the samples (3). In Pakistan, in the study of Zafar et al, 5 types of antibiotics were identified in the effluent discharged from two treatment plants into the river, whose concentrations are high in the limit of μg/L (30). Also, in a study in China, Beijing, 8 treatment plants were investigated and 14 out of 22 aimed antibiotics were identified, the maximum concentration of which was 3.1 μg/L in the influent and 1.2 μg/L in the effluent. The concentration of antibiotics in the sludge was 1.0×10^{-1} to 2.1×10^{4} μg/kg (23). Another study conducted in Iran reported antibiotics at concentrations of 1.6-10.7 μg/L in the effluents of treatment plants (19). It is worth mentioning that the concentration of antibiotics in the treatment plant's effluent is higher than their influent concentration in a number of studies (21,23,31,32). Another study conducted in Tehran, Iran, on the influents and effluents of WWTPs reported that out of 9 studied antibiotics, 7 antibiotics were detected in the samples (21).

It should be noted that all studies that have monitored antibiotics in WWTPs have been conducted before COVID-19 outbreak and increasing the consumption of antibiotics after the prevalence of COVID-19 (2,4) would also expand their concentration in WWTPs. In other study in Isfahan, Iran, by Gholipour et al during the COVID-19 pandemic (at the present study time), it was revealed that the antibiotics concentration in the WWTPs influent increased (33).

So this study aimed to identify and monitor the concentrations of the common antibiotics in Iran in the influent and effluent of WWTPs in Isfahan. The performance of the WWTPs processes on the removal of these compounds during the COVID-19 pandemic was also evaluated. Therefore, ampicillin and penicillin G antibiotics, which are among the most common antibiotics in Iran and Isfahan, were assessed and monitored (34,35). In addition to the high prescription of ampicillin and penicillin G, they are cheap in Iran, therefore, many patients buy and consume them arbitrary. Also, the flu which is a seasonal disease along with COVID-19 outbreak during the sampling period could result in the overuse of these two antibiotics.

Materials and Methods

Chemicals

In this study, the following analytical standard grade chemicals were purchased: The potassium salt of penicillin G (N99%), sodium salt of ampicillin (N99%), and sodium salt of phosphate (N99%) from Sigma-Aldrich (Steinham, Germany). Also, the Syringe filters were supplied from Membrane Solutions Company (USA). The Milli-Q water was supplied from SKY Company (Iran). The high-performance liquid chromatography (HPLC) grade acetic acid and acetonitrile (N85%) were supplied from Merck

(Darmstadt, Germany).

Studied antibiotics

A common and broad group of antibiotics are β-lactam antibiotics, which are used by more than 65% in the world. About 32.6% of antibiotics used in Iran pertain to the group of β-lactams (ampicillin, penicillin, amoxicillin) and according to studies, these are among extensively consumed arbitrary antibiotics in Iran (8). According to statistics provided by Isfahan University of Medical Sciences in 2020, ampicillin and penicillin are two of the most common β-lactam antibiotics used in public hospitals, and also, clinics in Isfahan. Therefore, due to the high consumption of these compounds, and also, the high probability of their existence in aquatic environments, especially wastewaters, they were investigated in this study (36).

Sampling sites

This study was conducted for two months (February to March, 2020) simultaneously in two municipal WWTPs in Isfahan along with the occurrence of COVID-19 pandemic worldwide. The geographical information system (GIS) map of the location of WWTPs is shown in Figure 1.

WWTP E, which covers a population of about 500 000 persons (2 modules 250 000 persons) and has average flow about 100 000 m^3/day. The treatment process in WWTP E is lagoon type and consists of anaerobic, aerobic and facultative lagoons. The facultative lagoons effluent transferred and reused. The treatment process diagram is shown in Figure 2-I.

WWTP S, which covers a population of about 900 000 persons (2 modules) and has an average flow of 130 000 m^3/day. The treatment process in WWTP S is activated sludge process. The effluent of secondary settling tanks is discharged into the river. The treatment process diagram is shown in Figure 2-II.

Sample collection procedure

In this study, a total of 52 samples were collected from two WWTPs with a share of 26 samples per WWTP. In each WWTP, 13 sample were taken from influent (after screening) and 13 samples from the final effluent. All sample bottle containers were initially washed with 10% nitric acid, and then, with distilled water (26). Sampling was performed in dark glass bottles and conveyed in cool box to the laboratory at 4°C (30,37).

Since the pH of the samples was almost in the neutral pH range (7.2-7.6), no pH adjustment was made.

Sample preparation and analytical method

Milli-Q water was used to prepare a standard solution of standard analytical grade antibiotics with a known concentration of about 1000 mg/L. In order to prevent long-term storage and decomposition of antibiotics, aluminum foil was used to cover the containers of samples, and then, stored at 4°C before use (30).

Before samples injection into HPLC, they were centrifuged at 4000 rpm for 5 minutes. Then, the isolated solutions were passed through a CA Syringe filter, and finally, injected into HPLC-UV.

Analysis techniques

Ampicillin and penicillin G were measured by HPLC (model: Jasco PU-2080, Tokyo, Japan) and equipped with a UV-VIS detector (UV-2075 plus), C18 column (150 × 4.6 mm, Germany), automatic injector (AS-2055 Plus) at a wavelength of 254 nm.

For the mobile phase, Milli-Q water, acetonitrile, NaH_2PO_4 1M, and acetic acid 1N were used at a ratio of 909:80:10:1 v/v. Table 1 shows the measurement method for each analytes.

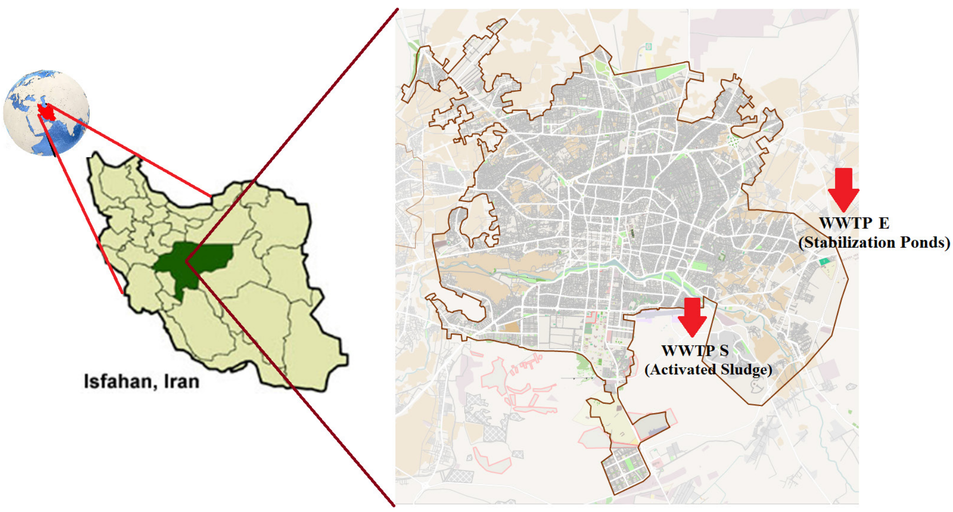

Figure 1. GIS map of the studied WWTPs location.

Table 1. Conditions set for measuring antibiotics using HPLC/UV, calibration equations and detection limits

Antibiotics	Wavelength (nm)	Retention time (min)	Calibration curves		LOD (µg/L)	LOQ (µg/L)	Recovery (%)
			Equation	R^2			
Ampicillin	254	5/125	y= 436.1106x+3316.9575	0.9952	55.5	168.18	96.4
Penicillin G	254	14/983	y= 91.342x +2013.9	0.9994	8	24.24	95.4

Method validation

Various parameters are used to confirm the validity and accuracy of the testing process. One of the parameters calculated in this study is the linear regression coefficient (R^2), the value of which in this study was above 0.99, indicating the high accuracy of this study. The next parameters used to ensure the accuracy of the study are limit of detection (LOD) and limit of quantification (LOQ). It should be noted that the measured concentrations should be above the range of these two parameters. LOD and LOQ are calculated using the following equations (Eqs. 1 and 2):

$$LOD = 3.3\left(\frac{standard\ deviation}{slope\ of\ the\ calibration\ curve}\right) \quad (1)$$

$$LOQ = 10\left(\frac{standard\ deviation}{slope\ of\ the\ calibration\ curve}\right) \quad (2)$$

Finally, the total method recovery was calculated using Eq. (3). Figure 3 shows the chromatogram diagram for the target antibiotics.

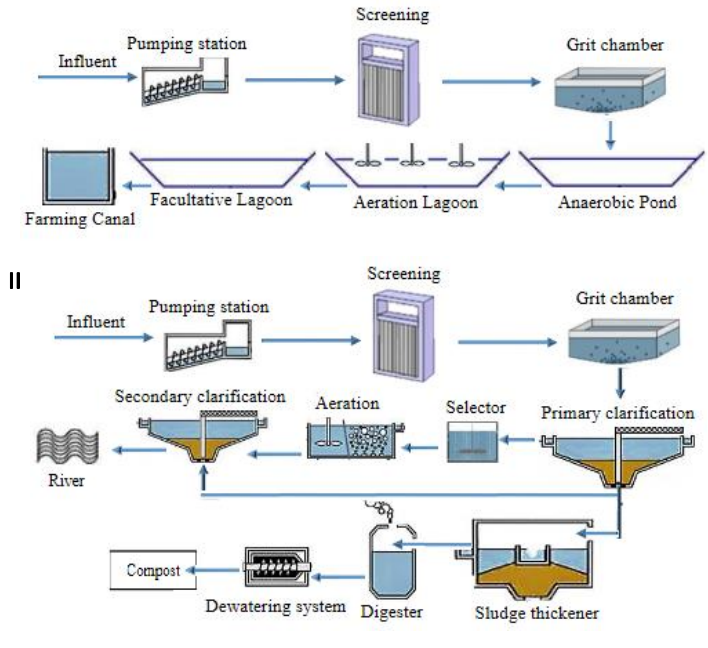

Figure 2. The flow diagrams of treatment processes in I- WWTP E and II- WWTP S.

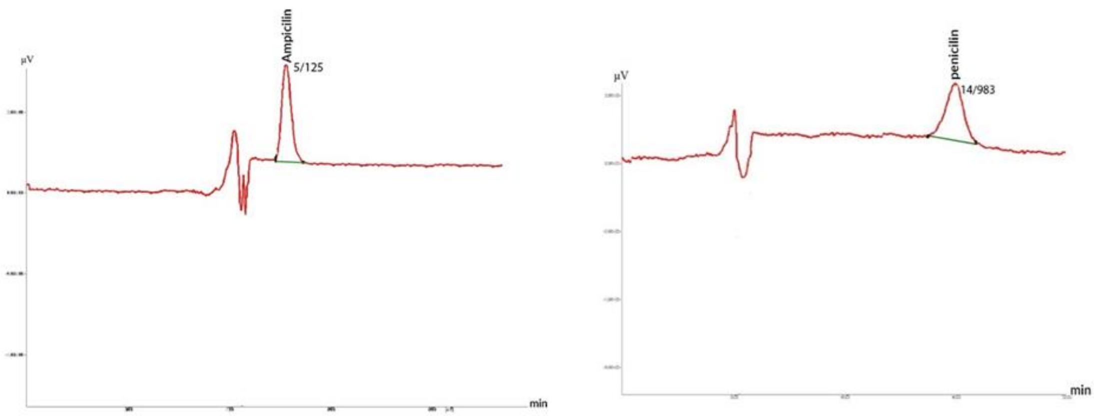

Figure 3. The chromatogram diagrams of target antibiotics.

$$Recovery(\%) = \frac{Real\,Concentration}{Artificial\,Concentration} \times 100 \quad (3)$$

Calculation of removal efficiency in WWTP

Equation (4) were applied to calculate the removal efficiency of antibiotics (38).

$$\text{Removal Efficiency}(\%) = \frac{C_0 - C}{C_0} \times 100 \quad (4)$$

Where C_0 is the inlet antibiotics concentration and C is the outlet antibiotics concentration.

In cases where the concentration of antibiotics in effluent samples was not quantified, due to their concentrations below the corresponding LOD, the value of $\frac{LOD}{\sqrt{2}}$ was considered in order to perform analysis (39).

Statistical analysis

SPSS software version 20 (40) was used for data analysis, and minimum, maximum, mean, average, and standard deviation were calculated. Kolmogorov-Smirnov (K-S) non-parametric test was applied to investigate the normality of the data. The Mann-Whitney U non-parametric test was applied to check the significant differences between the concentrations of antibiotics at the entrance of the two WWTPs. Spearman's method was also used to examine the data correlation.

Results

As mentioned in the previous section, the target antibiotics (ampicillin and penicillin G) were detected in all samples. The concentrations of target antibiotics are shown in Figure 4. Also, the removal efficiency of antibiotics in two treatment plants are presented in Figure 5.

Table 2 represents, minimum, maximum, mean, average, and standard deviation of ampicillin and penicillin G in WWTPs E and S.

For better comparison of the concentration of target antibiotics before and after the COVID-19 outbreak, the concentration of ampicillin and penicillin G which presented in different published articles and different

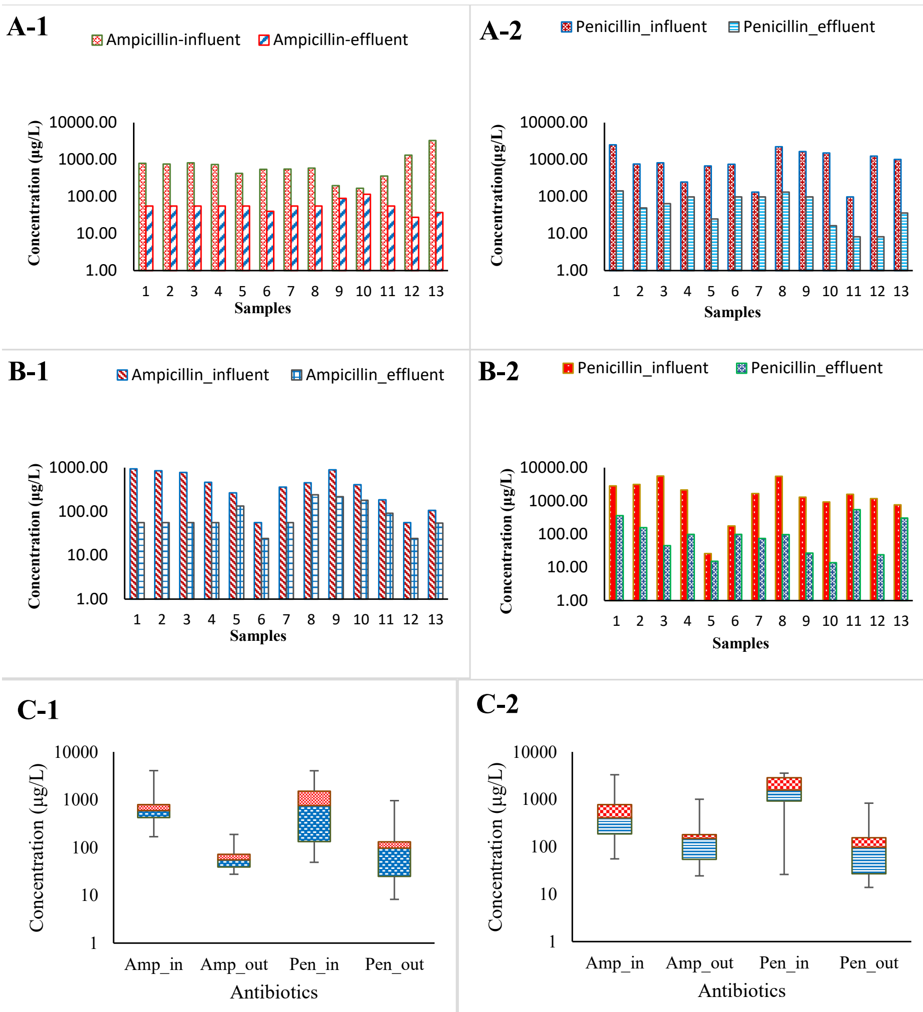

Figure 4. A-1: Concentration of ampicillin in WWTP E, A-2: Concentration of penicillin G in WWTP E, B-1: Concentration of ampicillin in WWTP S, B-2: Concentration of penicillin G in WWTP S, C-1: Box plot diagram of target antibiotics concentration in WWTP E, C-2: Box plot diagram of target antibiotics concentration in WWTP S.

environmental areas, are summarized in Table 3.

The results of K-S test showed that most of the data do not follow the normal distribution ($P<0.05$), so a non-parametric test should be used to analyze the data and the results are presented in Table 4.

The Spearman's correlation was applied to examine the correlation between the wastewater flowrate (Q), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total suspended solids (TSS), and antibiotics removal efficiency. The results are presented in Table 5. The desired parameters (Q, BOD_5, COD, TSS) were measured in the WWTPs laboratory and the results are summarized in Figure 6.

Table 6 shows the Spearman's correlation results between wastewater parameters (BOD_5, COD, TSS) and ampicillin and penicillin G removal efficiency in the two treatment plants.

Discussion

The aim of this study was to monitor ampicillin and penicillin G, which are among the most commonly used and prescribed antibiotics, in two municipal WWTPs of Isfahan during the COVID-19 pandemic.

The measured concentrations of antibiotics in WWTPs E and S are represented in Figure 4. The maximum, average, mean, minimum, and standard deviation

Table 2. Descriptive analysis of influents and effluent of target antibiotics

	WWTP E				WWTP S			
	Ampicillin (µg/L)		Penicillin G (µg/L)		Ampicillin (µg/L)		Penicillin G (µg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Maximum	3265.06	115.29	2522.37	142.08	935.84	240.48	5561.67	544.9
Mean	809.6	48.94	1050.54	52.89	447.1	90.31	2055.12	143.01
Median	588.12	39.24	824.52	36.23	406.59	39.24	1568.87	96.58
Standard deviation	±796.44	±24.25	±761.43	±49.27	±322.39	±75.91	±1788.08	±162.59
Minimum	168.17	39.24	97.7	5.66	55.97	39.24	26.04	13.88

Table 3. Target antibiotics concentration in different *aquatic* environment before and after the COVID-19 pandemic

Antibiotics	Country and city	Source of detection	Concentration (µg/L)	Time of detection	Reference
Ampicillin	Nairobi County- Kenya	Influent	0.2	Before COVID-19	(5)
	Nairobi County- Kenya	Effluent	0.2	Before COVID-19	(5)
	Nairobi County- Kenya	Water Pond	0.18-0.22	Before COVID-19	(5)
	Nairobi County- Kenya	River	0.1-0.24	Before COVID-19	(5)
	Nairobi County- Kenya	Effluent from laboratory	0.18	Before COVID-19	(5)
	Rawalpindi and Islamabad- Pakistan	wastewater	12-32570	Before COVID-19	(30)
	European wastewater treatment plants	Effluent	0.0681 & 0.0994	Before COVID-19	(3)
	Greece	wastewater	0.498	Before COVID-19	(17)
	Iran-Isfahan-WWTP E- Stabilization ponds	Influent	809.6	After COVID-19	This study
	Iran-Isfahan-WWTP E- Stabilization ponds	Effluent	48.94	After COVID-19	This study
	Iran-Isfahan-WWTP S- Activated sludge	Influent	447.1	After COVID-19	This study
	Iran-Isfahan-WWTP S - Activated sludge	Effluent	90.31	After COVID-19	This study
Penicillin G	Nairobi County- Kenya	Influent	< 0.22	Before COVID-19	(5)
	Nairobi County- Kenya	Effluent	< 0.22	Before COVID-19	(5)
	Nairobi County- Kenya	Water Pond	< 0.22	Before COVID-19	(5)
	Nairobi County- Kenya	River	< 0.22	Before COVID-19	(5)
	Nairobi County- Kenya	Effluent from laboratory	< 0.22	Before COVID-19	(5)
	Iran-Tehran	Influent	0.0132-0.03637 & 0.034-0.06589	Before COVID-19	(21)
	Iran-Tehran	Effluent	0.01713 & 0.007553-0.03118	Before COVID-19	(21)
	Iran-Tehran	River	0.01555	Before COVID-19	(21)
	Iran-Isfahan-WWTP E- Stabilization ponds	Influent	1050.54	After COVID-19	This study
	Iran-Isfahan-WWTP E- Stabilization ponds	Effluent	52.89	After COVID-19	This study
	Iran-Isfahan-WWTP S- Activated sludge	Influent	2055.12	After COVID-19	This study
	Iran-Isfahan-WWTP S- Activated sludge	Effluent	143.01	After COVID-19	This study

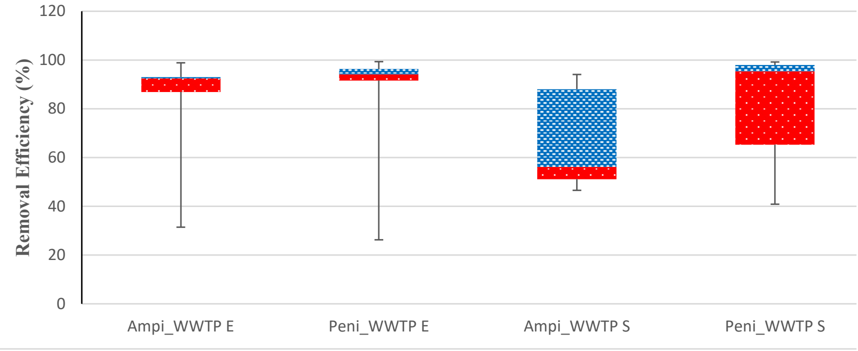

Figure 5. Removal rate of target antibiotics in WWTPs E and S.

of target antibiotics for two studied WWTPs at the influent and effluent are shown in Table 2. The average concentrations of ampicillin and penicillin G in the influent and effluent of WWTP E, were 809.6 ± 796.44, 48.94 ± 24.25, 1050.54 ± 761.43, and 48.94 ± 24.25 µg/L, respectively. Also, the average concentrations of ampicillin and penicillin G in the influent and effluent of WWTP S were 447.1 ± 322.39, 90.31 ± 75.91, 2055.12 ± 1788.08, and 143.01 ± 162.59 µg/L, respectively.

According to the information given in Figure 4-A1 and Table 2, in WWTP E, the maximum and minimum concentrations of ampicillin in the influent were 3265.06 and 168.17 µg/L and in the effluent were 115.29 and 39.24 µg/L, respectively. Also, according to Figure 4-A2 and Table 2, in WWTP E, the maximum and minimum concentrations of penicillin G in the influent were 2522.37 and 97.7 µg/L and in the effluent were 142.08 and 5.66 µg/L, respectively.

The maximum and minimum concentrations of ampicillin in WWTP S, were 935.84 and 55.97 µg/L in the influent and 240.48 and 39.24 µg/L in the effluent, which are shown in Figure 4-B1 and Table 2. Also, according to Figure 4-B2 and Table 2, the maximum and minimum concentrations of penicillin G in the influent were 5561.67 and 544.9 µg/L and in the effluent were 26.04 and 13.88 µg/L, respectively.

Based on the results of this study in two WWTPs, the highest concentration was related to the penicillin G antibiotic, which is shown in Figure 4-C1 and C2. In the influent of WWTPs E and S, the median concentrations of ampicillin and penicillin G, were 588.12, 824.52, 406.59, and 1568.87 µg/L, respectively. The results show that penicillin G is more consumed than ampicillin in Isfahan.

Another study in Kenya, the average concentrations of ampicillin and penicillin G in the influent and effluent of the WWTP were reported to be in 0.22 and 0.18-1.24 µg/L, respectively (5), which were higher in the present study. Also, in a study conducted in Pakistan, ampicillin antibiotic was detected in wastewater samples collected from 15 points in the city and its concentration was reported to be 0.012 to 32.570 µg/mL (30), which is consistent with the results of the present study. In other study, it was reported that in the effluents of 13 treatment plants in European countries, the concentration of ampicillin in two treatment plants was reported 99.4 and 68.1 ng/L, respectively (3). In other study performed in Greece, the concentration of ampicillin in the effluent of the WWTP was reported to be 498 ng/L (17). Another study reported that in the effluent of two WWTPs in Tehran, the concentration of penicillin G was about 7.50

Table 4. The results of Mann-Whitney U test for antibiotics comparison in WWTPs E and S

Parameters	Ampicillin	Penicillin G
Total N	26	26
Mann-Whitney U	59.000	118.000
P-value	0.204	0.091

Table 5. Spearman Correlation between wastewater parameters in influent of WWTPs E and S

Parameters	WWTP E		WWTP S	
	Ampicillin	Penicillin G	Ampicillin	Penicillin G
Ampicillin $_{in}$	1.000	-0.088	1.000	0.753**
Penicillin G $_{in}$	-0.088	1.000	0.753**	1.000
Q $_{in}$	-0.063	0.188	0.742**	0.597*
$BOD_{5\,in}$	-0.007	0.691**	0.182	0.099
COD $_{in}$	-0.007	0.691**	0.610*	0.566*
TSS $_{in}$	-0.262	0.248	-0.140	-0.146

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

Table 6. Spearman's correlation among removal efficiencies in WWTPs E and S

Parameters	WWTP E		WWTP S	
	R_ Ampicillin	R_ Penicillin G	R_ Ampicillin	R_ Penicillin G
R_ Ampicillin	1.000	-0.560*	1.000	0.055
R_ Penicillin G	-0.560*	1.000	0.055	1.000
R_BOD_5	0.242	0.054	-0.075	0.332
R_COD	0.248	0.007	0.421	-0.129
R_TSS	-0.617*	0.268	0.407	0.231

*. Correlation is significant at the 0.05 level.

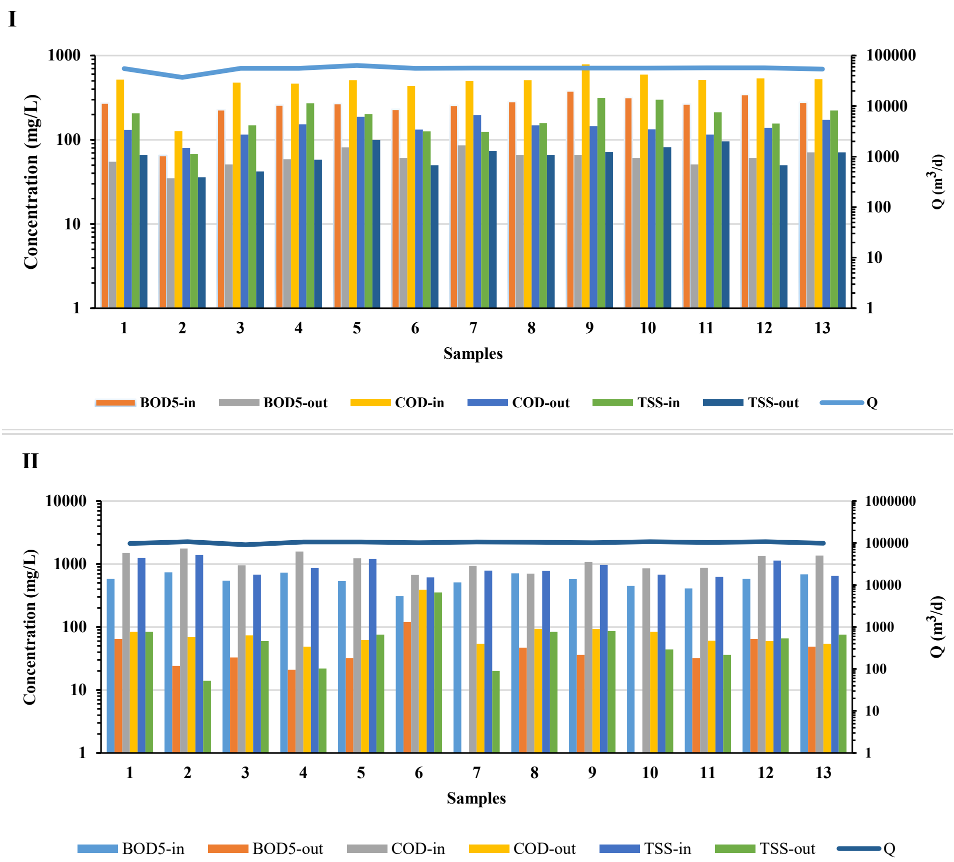

Figure 6. Wastewater treatment plants main parameters, I: WWTP E and II: WWTP S.

and 20.04 ng/L (21).

Comparing the results of this study with similar studies, it was found that the concentration of penicillin G and ampicillin in the municipal WWTPs located in Isfahan city was higher than that reported in other published areas. These results could indicate that the consumption of antibiotics in Isfahan and Iran may be higher than other regions of the world. Contrastingly, along with the COVID-19 pandemic, the use of these antibiotics and antibacterial drugs has increased (1,2). Moreover, in a study conducted in Iran, it was revealed that the antibiotics concentration in the effluent of municipal WWTPs increased during COVID-19 pandemic. In addition, since hospitals and patients are scattered throughout the city of Isfahan, most of the hospital wastewater influent is related to WWPT S (33), which is consistent with the results of this research. In Table 3, the results of comparison of the concentration of ampicillin and penicillin G in different water environments before and after the COVID-19 pandemic are presented.

In terms of removal efficiency, penicillin G with an average removal efficiency of 89.8 ± 19.42% in WWTP E and 82.76 ± 21.85% in WWTP S had higher removal efficiency, and ampicillin with an average removal efficiency of 86.22 ± 19.84% in WWTP E and 66.85 ± 24.88% in WWTP S had lower removal efficiency in two studied WWTPs. Figure 5 shows removal efficiency of target antibiotics in WWTPs E and S. As shown in Figure 5, WWTP E had higher removal efficiency of antibiotics than WWTP S.

Although a number of studies have shown that pharmaceuticals are well removed by the activated sludge process (41-44), the findings of the present study showed that WWTP E by aeration lagoon/stabilization pond system have eliminated antibiotics better than WWTP S. The reason can be due to the longer retention time and larger pond's area that leads to more exposure to sun's UV that eliminate antibiotics better than activated sludge system (WWTP S). As the ability of each treatment system to remove antibiotics is different, therefore, municipal WWPTs are not able to remove all pollutants. In studies, the removal efficiency is usually less than 80% and few treatment plants with 100% efficiency are observed (19).

Shi et al reported about 57 to 95% of the removal of antibiotics in municipal WWTPs in China (13). Although the concentration of antibiotics in the effluent was high, but it seems that the treatment plants have shown a high ability to remove antibiotics. In a study by Mirzaei et al the removal efficiency of penicillin G was reported 59.4% and 50.43% (21), so it is observed that both studied treatment plants with removal efficiency above 80%, had removed penicillin G relatively well.

According to the results of Mann-Whitney U test shown in Table 5, there is no significant difference between the valuations of ampicillin and penicillin G in each of the two WWTPs.

Based on the results of Spearman's correlation shown in Table 5, there are no significant relationships between the main parameters of wastewater and the concentration of ampicillin antibiotic in WWTP E, but the concentration of penicillin G is correlated with the wastewater parameters.

In WWTP S, a significant correlation was observed between the influent concentration of penicillin G and ampicillin with each other, and also, with the parameters of influent discharge (Q_{in} and COD_{in}).

The results of Spearman's rank-order correlation coefficient were represented in Table 6. The findings in WWTP E and S indicate that the correlation between antibiotics removal efficiencies and the main parameters of wastewater (BOD, COD, TSS) removal efficiencies is not significant. However, according to Table 6, the correlation between the removal efficiencies of the two target antibiotics was significant in WWTP E, but it was not significant in WWTP S. This issue could be due to the difference in the process of two WWTPs, one of which is activated sludge and the other is stabilization ponds.

Conclusion

In the present study, the concentrations of two β-lactam antibiotics, ampicillin and penicillin G, were monitored and measured in two WWTPs in Isfahan. The results revealed that the concentration of these two antibiotics in the influent and effluent is higher than that reported in other published areas. The first reason for this issue could be the excessive arbitrary consumption of these antibiotics in Iran and Isfahan. The next reason that has led to higher concentrations in wastewater is the further use of these antibiotics due to the COVID-19 pandemic. Also, due to the reuse of the effluent of WWTPs, it is necessary to pay special attention to remove these antibiotics from the effluents. The results of statistical analysis also showed that there is no difference between the removal efficiency of other wastewater parameters such as BOD_5, COD, and TSS and the removal efficiency of antibiotics in the studied WWTPs and they are independent of each other. But in WWTP with stabilization ponds process, the removal efficiencies of the two antibiotics were significantly correlated.

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Ethical issues

The present study was approved by Isfahan University of Medical Sciences (Ethical Code: IR.MUI.RESEARCH.REC.1398.020).

Competing interests

The authors agree to publish this article. In addition, the ethical principles of research have been observed in this study.

Authors' contributions

HMA contributed to supervision, conceptualization, review and editing. MS contributed to investigation, writing original draft, formal analysis, laboratory experiments. KE contributed to methodology, experimental advisor. FM performed data investigation, statistical analysis, review and editing. SG conducted English review and editing.

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