

Common Antibiotics in Wastewater of Sina and Besat Hospitals, Hamadan, Iran

Reza Shokoohi^a, Mostafa Leili^a, Abdollah Dargahi^{b*}, Yaser Vaziri^b, Razieh Khamutian^b

^aDepartment of Environmental Health Engineering, School of Health, Hamadan University of Medical Sciences, Hamadan, Iran.

^bStudent Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran.

*Correspondence should be addressed to Mr. Abdollah Dargahi, Email: a.dargahi29@yahoo.com

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Background & Aims of the Study: Antibiotics utilize with the aim of improving human, animal and plant health; also, for treating infections caused by pathogenic bacteria. When these compounds introduce into ecosystems, could affect the microbial community and jeopardize human health. Therefore, the aim of present study is to determination of the presence of six common utilized antibiotics (Amoxicillin, Ciprofloxacin, Erythromycin, Sulfamethoxazole, Imipenem and Cefixime) in wastewater effluent of Sina and Besat hospitals in Hamadan, Iran.

Methods: This study was descriptive-applied type. Sampling was conducted as compound samples from nearest manhole to hospitals containing toilets wastewater. Samples were transferred into the laboratory in dark conditions at 4°C and after centrifugation, passed through 0.45µ filters. Then, samples were extracted up to 24h and kept at -18°C until analysis. Prepared samples measured via HPLC (High Pressured Liquid Chromatography) system.

Results: Data analysis indicated the presence of large and unfavorable amounts of three antibiotics including Amoxicillin, Cefixime and Imipenem in hospital wastewaters of Sina hospital, so that; mean concentration of identified antibiotics for Sina hospital was 5.86, 10.85 and 25.53 µg/L, respectively. Also, none of the most common antibiotics were observed in wastewater from Besat hospital.

Conclusion: Results showed that great difference between measured antibiotics from hospitals' wastewater is due to intensive fluctuations in quantity and quality of effluent wastewater from hospitals. Generally, identified amounts of three antibiotics (Amoxicillin, Cefixime and Imipenem) represent improper status of presence of residuals in Sina hospital's effluent wastewater which mixing this flow in urban wastewater; this flow could result in increasing concerns about entering antibiotic compounds into environment.

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Background

Nowadays, drugs are considered as very important and integral part of modern life and utilized for treating human and animal illnesses. The presence of drugs in the environment is one of the important issues in the world which just recently some of developed countries like USA, England, Germany and Italy commenced to study the negative impacts of these pollutants on the environment. Recently, a few scientists interested in finding the fate of these drugs by

wide volume, thousands distribution points and their impacts on living organisms and the environment (1). Meanwhile, antibiotics as most important part of drugs have a considerable contribution in protection of human and animal against microbial infections (2). In other word, antibiotics are most successful drugs in human therapy (3). These drug classes have great utilization in poultry and aquiculture farms for increasing growth (4). Global application of antibiotics estimated at 100000 to 200000 tons, annually (5). Antibiotics have stable impacts on environment

and great importance (6). Often, applied antibiotics in human and animal were not metabolized and 90 percent remains active after excretion. Therefore, the main part of these compounds or their metabolites excreted from the body via urine and faeces in unchanged or partially changed form, entered into wastewater networks (7-8) and eventually into wastewater treatment plants. Some of these compounds or their metabolites were not totally removed in wastewater treatment systems, so, considerable concentrations had been found in effluents from wastewater treatment plants. Disposal of the wastewater of treatment plant into environment leads to presence of these compounds in different parts of aqueous environment, so that antibiotics in ng/L to µg/L ranges recognized in rivers (9), lakes (10), groundwater (11) and soils (12). Therefore, it can be concluded that, in best situation, 30000 tons and in worst one 180000 tons active antibiotics introduce into wastewaters annually and eventually to receptor waters (13).

In Heidari et al study (2014) about the qualitative analysis of five antibiotics (Ampicillin, Oxytetracycline, Enrofloxacin, Tylosin and Ciprofloxacin) in wastewater and effluent from a municipal wastewater treatment plant which is located in the central region of Iran, just ciprofloxacin and ampicillin were detected in wastewater and effluent (14). Various authors know wastewaters as favorable habitat for many bacteria for being resistance against various antibiotics. Hospital wastewaters have greater importance (15).

Aims of the study: Since there was no study about investigating and identifying antibiotics in wastewaters in Hamadan city, the present study was conducted with the aim of determination of the presence of six common utilized antibiotics (Amoxicillin, Ciprofloxacin, Erythromycin, Sulfamethoxazole, Imipenem and Cefixime) in wastewater effluent of Sina and Besat hospitals in Hamadan, Iran.

Materials & Methods

The study was descriptive-applied which conducted in Sina and Besat hospitals for three months (from April to June, 2016). For this purpose, monthly two Composite Samples were taken from hospitals' raw wastewater, so, 12 Composite samples collected and concentration of six antibiotics were measured in each sample. Also, in order to assure the reliability of results, some ambiguous analysis repeated three times, so, considering the replications, 216 analysis were conducted.

Chemicals and reagents

In the present study, common and most utilized antibiotics were identified by referring to hospitals, important drug stores and food and drug deputy of Hamadan University of medical sciences. According to these surveys, most utilized antibiotics in Hamadan were Amoxicillin from Penicillin, Cefixime from third generation Cephalosporin, Ciprofloxacin from Flouroquinolone, Erythromycin from macrolides, Sulfamethoxazole from sulfonamides. Standards of Amoxicillin, Ciprofloxacin, Erythromycin, Sulfamethoxazole, Imipenem and Cefixime antibiotics purchased from Sigma Aldrich Co., Germany. HPLC grade methanol and Ultra-pure water purchased from Merck Company (Germany). SPE cartridges (by 200 mg absorbent amount and 6 ml volume) prepared from Thailand Corporation. Cellulose acetate filter with pore size of 0.45 µm and cellulose acetate syringe filter with pore size of 0.2 µm were utilized. In addition, analytical grade sulfuric acid and Di-sodium ethylene-di-amine tetra acetate (Na₂EDTA) purchased from Merck Company, Germany. The Main solution for each antibiotic separately prepared in a mixture of methanol and water (volume ratio of 1:1) at 1 mg/L concentration and kept in freezer at -10°C. Mixed standard solutions from 10 to 200 µg/L concentration range prepared, using stock solutions in a mixture of methanol and HPLC grade water (volume ratio of 3:1). All standard

solutions (including stock and work solutions) stored at capped 50µL volumetric balloon at -10°C.

Samples:

Sampling location was the nearest manhole to Sina (infectious) and Besat (non-infectious) hospitals containing sanitary wastewater effluent. Composite sampling was conducted to decrease qualitative fluctuations in effluent wastewater from hospitals. Daily, two sets of 1 liter samples, first at 11 Am and the second at 3 Pm, harvested for one hour at 10 min time intervals and transferred to laboratory in glass flasks at -4°C and mixed in laboratory. In the laboratory, regarding to large amounts of suspended materials in samples, especially in samples collected from raw wastewaters, each sample initially centrifuged at 4000 rpm for 40 min. Then, samples filtered through 0.45µm cellulose acetate filter. pH adjusted at 3 by the addition of 3mol sulfuric acid. In order to remove interferences of metal ions in the extraction process and analysis, 0.2g Na₂EDTA added to filtered acidic samples. Ultimately, samples extracted up to 24h and kept at -18°C until analysis.

Solid phase extraction (SPE):

Solid phase extraction process conducted, using SPE cartridges by 200 mg absorbent and volume of 6ml. Air was pumped from the vacuum flask through vacuum pump and cartridges attached to the inner space of flask, using a 1ml pipette. Cartridges operated via passing over of 4ml methanol and then 6ml deionized water. 100 ml wastewater sample by

pH of 2.8 to 3 passed through cartridge by 5-8 ml/min flow rate under vacuum of 7-9 inches mercury, using an extractive manifold. In this condition, having regard to absorbent and antibiotic properties, interested compounds separated from wastewater matrix and remained in SPE absorbent. Then, 10 ml Ultra-pure water passed away from cartridges for leaching and cartridges dried by air for 5 min. Finally, residual analytes transferred to glass tubes, using 10 ml methanol. Extracts concentrated up to drying under nitrogen flow and recovered to 250 ml volume in a mixture of ultra-pure water and methanol solvent for leaching (9:1 ratio). Lastly, extracts are filtered through the cellulose acetate syringe filter by 0.2 µm pore size and 4mm in diameter, transferred to brown vials and kept at -15°C until analysis. In order to measuring antibiotics in samples, HPLC utilized. Figure 1 indicated schematic presentation of solid phase extraction for studied antibiotics.

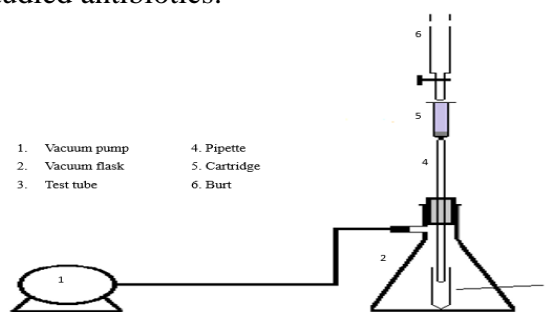


Figure 1: schematic presentation of solid phase extraction for studied antibiotics

Table 1) Chromatographic conditions

Antibiotics	Injection volume (µl)	Flow velocity (ml/min)	Column temperature(°C)	Wavelength (nm)
Cefixime	25	0.9	20	290
Amoxicillin	25	0.9	20	250
Imipenem	25	0.8	20	260
Sulfamethoxazole	25	1	25	235
Ciprofloxacin	25	1	20	279
Erythromycin	25	1	25	210

Measurement via HPLC:

Due to low accuracy of simultaneous analysis methods, in the present study, special and

separate methods utilized for measuring antibiotics. Utilized chromatography system Agilent 1200 (made in USA) equipped by

Autosampler. Extracted liquid simultaneously passed through C18 column (ODS-3 250×4.6mm, 5µm) by mobile phase of acetate (pH 4) and acetonitrile (89:11 ratio) along with 0.1 percent formic acid at 30°C and flow rate of 0.8 ml/min. Chromatographic conditions required for each antibiotic presented in Table 1. It should be noted that sample prior to injection to the column, filter via 0.2µm syringe filters.

Results

Assessing the validity of chromatography method:

In order to calculating linearity index, different concentrations of each antibiotic must be prepared. After analysis and obtaining the results, calibration curve plotted and regression coefficient index (R^2) was calculated for achieving the linearity. It should be noted that, in the present study for Imipenem, amoxicillin and Cefixime, totally 7 measurement points at 1-80, 1-70 and 1.5-50 µg/l concentrations, respectively, utilized for plotting calibration curve and determination of linearity index.

Calibration curves with acceptable linearity index obtained about 0.9997 for Amoxicillin, 0.9992 for Cefixime and 0.9996 for Imipenem which represent the accuracy of calibration curves and determined concentrations.

Antibiotic concentrations in hospital wastewaters:

According to Table 2, results of data analysis indicate the presence of large and unfavorable amounts of three antibiotics including Amoxicillin, Cefixime and Imipenem in Sina hospital's wastewater, so that, the mean concentration of Imipenem in wastewaters of Sina hospital had the highest concentration (25.53µm/l). Also, according to Table 3, none of the most common antibiotics were observed in wastewater from Besat hospital. Comparison of identified antibiotics concentrations in Sina hospital presented in Fig. 2.

As shown in Fig. 1, the highest concentration was associated to Imipenem antibiotic in March (28.26µg/l). Also, samples from out of the peaks of Amoxicillin, Cefixime and Imipenem in wastewater samples have been compared with standard peak and shown in Fig. 3-5.

Table 2) concentrations of studied antibiotics in hospital wastewater of Sina hospital in Hamadan

Month	Antibiotics					
	Erythromycin	Sulfamethoxazole	Ciprofloxacin	Imipenem	Cefixime	Amoxicillin
April	ND	ND	ND	27.4	12.4	5.8
	ND	ND	ND	29.12	11.73	6.5
May	ND	ND	ND	26.63	10.87	7.91
	ND	ND	ND	25.16	8.6	5.85
June	ND	ND	ND	19.89	10.55	3.98
	ND	ND	ND	24.96	10.96	5.17
SD± Mean	-	-	-	25.53±2.88	10.85±1.18	5.86±1.2

Table 3) concentrations of studied antibiotics in hospital wastewater of Besat hospital in Hamadan

Month	Antibiotics					
	Erythromycin	Sulfamethoxazole	Ciprofloxacin	Imipenem	Cefixime	Amoxicillin
April	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND
May	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND
June	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND
SD± Mean	-	-	-	-	-	-

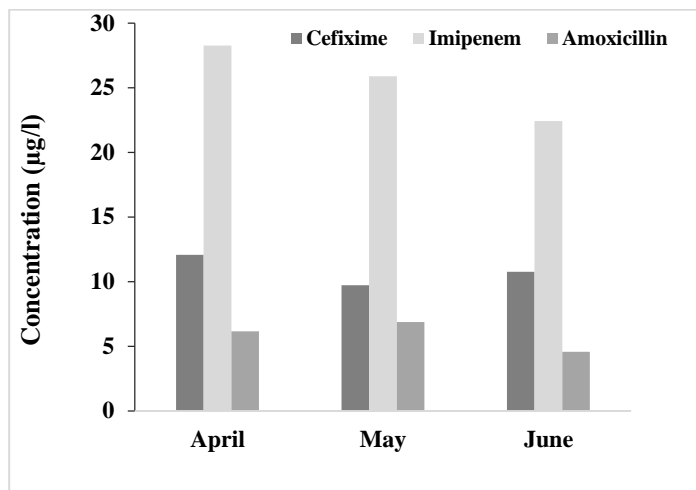


Figure 2) Comparison of identified antibiotics in Sina hospital

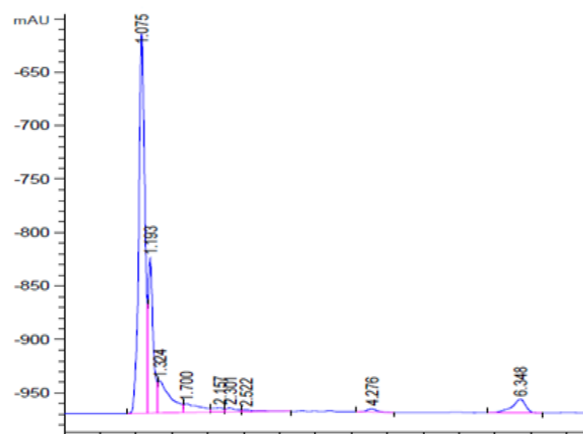


Figure 3) Output peak sample for Amoxicillin antibiotic

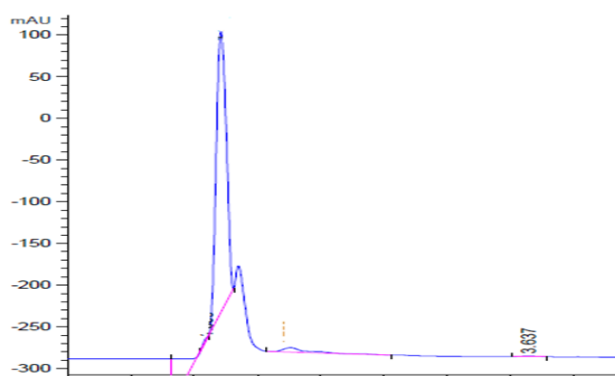


Figure 4) output peak sample for Imipenem antibiotic

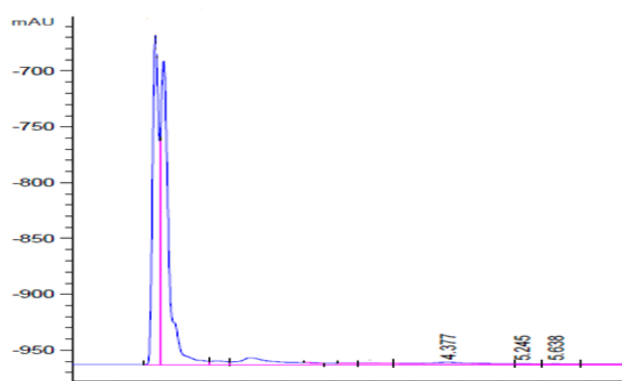


Figure 5) output peak sample for Cefixime antibiotic

Discussion

Concentration of antibiotics in hospital wastewater:

In the present study, among studied antibiotics for Sina and Besat hospitals, only three antibiotics including amoxicillin, imipenem and Cefixime identified in effluent wastewater of Sina hospital and there was no recognized antibiotic in Besat hospital wastewater effluent. Having regard to this point that Besat hospital is an infectious hospital, antibiotic application in this hospital is higher than other one. Imipenem had the highest concentration. Results showed that β -lactams which include penicillin, cephalosporin, mono-bactams and carbapenems, have the highest application rate

for human communities. The share of these antibiotics is almost 50 to 70 percent of total antibiotic applications (16,17). Imipenem is an antibiotic from carbapenem group which has the widest range and was considered as a last defense line against antibiotic resistant organisms.

The mean concentration of recognized antibiotics including amoxicillin, Cefixime and imipenem in Sina hospital wastewater were 5.86, 10.85 and 25.53 $\mu\text{g/L}$ which great difference among measured amounts from hospital wastewater could be due to intensive qualitative and quantitative fluctuations in wastewater effluent. So that, the highest amounts for these antibiotics from Sina hospital were 7.91, 12.4 and 29.12 $\mu\text{g/L}$, respectively

and the lowest amounts were 3.98, 8.6 and 19.89 µg/L, respectively. Lindberg et al. (2004) studied antibiotics in hospital wastewater at different time in day and night indicated this fact that wastewater effluent at different times had considerable differences (18). Dehghanzadeh et al. (2014) showed that in hospital wastewaters of Tabriz, mean and maximum concentration of imipenem in hospital effluent were 8.7 and 22.4 µg/L, respectively, which are in consistent with the present study (19).

Results of Ghafouri et al. (2014) who measured amoxicillin concentration in three selected hospitals in Bojnourd town on April, May and June months showed that, the highest antibiotic amount observed in Imam Ali (AS) on June by 234.4 µg/L and the lowest amount obtained in Bentolhoda hospital on April by 24.01 µg/L. In June, antibiotic concentration for all of three hospitals reported as zero and BentolHoda hospital had zero amount on May. Data dispersion in the present study could be attributed to the dispersion of drug consumption type and Amoxicillin application time in hospitals. Also, data dispersion could be due to randomized application of drugs in hospitals for laboratory and treatment uses (20). In the present study, the highest amount of recognized antibiotic for Cefixime and imipenem antibiotics observed on March (12.06 and 28.26 µg/L, respectively) and for amoxicillin on April (6.88 µg/L). Rossmann et al. (2014) studied various antibiotics in urban raw wastewater and found that highest and lowest amount for Amoxicillin was 1240 and 0 ng/L, for Sulphometaxasol was 2204 and 12 ng/L and for Ciprofloxacin was 1570 and 78 ng/L, respectively (21). In the present study, there were no Sulphometaxasol and Ciprofloxacin antibiotics in both studied hospitals. In a study, Pena et al. (2010) investigated the presence of antibiotics in hospital wastewater and showed that tetracycline amount in hospital wastewater was in 6 to 531.7 µg/L range and the highest antibiotic concentration observed in the Spring

season (22). Also, the results of ThiQuynh Lien et al. showed that the mean concentration of Ciprofloxacin antibiotic in hospital raw wastewater obtained as 42.8 µg/L (23).

In the present study, there was no Ciprofloxacin antibiotic in both studied hospitals. At the current situation, recognized amounts of all three antibiotics represents inappropriate status of present of residuals in wastewater effluent of Sina hospital; so, mixing these flows with urban wastewater flow could cause increasing concerns about introducing antibiotics in environment. On the other hand, the impact of these residuals on wastewater treatment processes could not be ignored. As a study in Hong Kong (2008) indicated, introduced antibiotics into wastewater treatment facilities caused disturbing in biological processes, especially interferences in algal growth in fixation ponds, in which the highest sensitivity had been for Amoxicillin (24).

Ultimately, it can be said that residuals of utilized antibiotics in aqueous environments not only considered as an environmental danger, but also imposes more treating-investigating costs for discovering new antibiotics in order to treating resistant infections. According to the statistics of antibiotic application in Iran, the presence of higher concentrations of antibiotics in hospital and urban wastewaters as well as lower amounts in surface, groundwater and drinkable water could not be surprising.

Conclusion

It can be concluded that the residual concentration of three antibiotics Cefixime, Amoxicillin and Imipenem in wastewater of infectious diseases hospital (Sina) were inappropriate, since these antibiotics were identified in all samples which were taken from Sina hospital during three months of study. Discharging of effluents from this hospital to Municipal wastewater stream can increase concerns about antibiotics in the environment.

Here, there are strategies for dealing with introduction of drugs, especially antibiotics into environment, including: applying management strategies for reducing antibiotic uses, giving information and making aware physicians and specialists about correct and rational use of antibiotics and no excessive prescription of antibiotics and in un-essential cases, culturally work about decreasing self-pharmaceuticals by peoples and also applying administration strategies for removing antibiotics and prevention of introducing them into environment like complete treatment of hospital wastewater.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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