Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2018.0402.07**

A QUALITATIVE SURVEY OF ANTIBIOTICS IN SEWAGE FROM HOSPITALS AT KOTA (RAJASTHAN)

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ABSTRACT: Antibiotics are medicines that inhibit the growth of or destroy bacteria. Inappropriate disposal of the drugs not only contaminate the environment, but also brings chances for development of antibiotic resistance strains within our surroundings. In this study the contamination levels of five antibiotics, namely Ampicillin (AMP), Cefadroxil (CEF), Cloxacilin (CLO), Ciprofloxacin (CIP) and Ofloxacin (OFL) were identified in samples of sewage of the three hospitals in Kota. HPLC with a VWD detector, C-18 column, and solid-phase cartridges were used to analyze antibiotic residues. The concentration of antibiotics was calculated in mg/L. The range of antibiotics concentration were CEF (2.16mg/L) > CIP (0.90mg/L) > AMP (0.83mg/L) > OFL (0.20mg/L) > CLO (0.18mg/L). In this study, the concentration of CEF (2.16mg/L) in the sewage was high. High values of concentration indicate the presence of comparatively large amount of antibiotic due to (i) high dose consumption and (ii) 60% of drug eliminates unchanged and this is a matter of concern in terms of its wider public health impact. The contamination level was much higher in sewage samples as it is the primary source of antibiotics entering into the environment.

KEYWORDS: Antibiotics, Sewage water, Solid Phase Extraction, HPLC, Antibiotic resistance and environment.

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1. INTRODUCTION

Antibiotics are becoming progressively questionable contaminants of water sources, especially in surface and ground water, which are located in the area of hospitals, agricultural land or industries. The term antibiotics, means "against life", or against microbes [1]. Antibiotics are the substances which produced by some microorganism that may either kill or inhibit the growth of bacteria with doing little or no harm to human cells. Antibiotics introduced as a medicine in the early 20th century [2]. Antibiotics are used extensively in human and veterinary medicine [3]. Chemical substances are used as human medicines and in agriculture and animal culture, in which antibiotic are one of the most important groups of common pharmaceuticals in our daily lives [4]. The efficacy and easy approach of antibiotics tended to overuse [5] of antibiotics, which resulted bacteria to develop resistance [6, 7]. Many studies showed that the antibiotic present in hospital effluent depends upon the volume of antibiotic prescriptions [8]. After the administration to humans, their metabolites are excreted into the sewage [9]. Wastewater treatment plants (WWTPs) are not designed to completely remove antibiotics, and consequently they are released into water resources. Moreover, antibiotics can pass through all natural filtrations and reach ultimately into drinking water due to their high water solubility and often poor degradability [10]. The regular use of antibiotic contaminated water can have many adverse effects on human health, including acute and chronic toxicity. Because antibiotics are designed to interfere with biological systems, their extended exposure can be harmful even in low concentrations [11-13]. They can also have toxic effects on the animals and aquatic lives. Increased use and exposure to antibiotics or improper use of antibiotics over the last few decades has increased bacterial resistance against them.

1.1. Fate of Antibiotics in the Environment of Kota

To my knowledge, this was the first study has been done in Kota region. Only the government hospitals are having sewage treatment plants, whereas the private hospitals, which are surrounded, by urban populations and are located in different areas of Kota and none of these hospitals have a treatment facility for the removal of antibiotics and other pharmaceutical compounds from their sewage. Usually wastewater from hospitals directly drains into the sewage system that in turn enters the canals used for crop irrigation. On the other hand, some amount of wastewater reach to ponds of stagnant water. These ponds are habitat of aquatic life and a source of drinking water for animals. So the most immediate need is to develop an easy, economic, reproducible, precise, and accurate method of quantification of residual levels of antibiotics. Ciprofloxacin (CIP) and ofloxacin (OFL) from fluoroquinolones, ampicillin (AMP) and cloxacillin (CLO) from penicillin, cefadroxil (CEF) from Cephalosporins were selected for this study. The selection of antibiotics was based on the following aspects: (1) use of antibiotics (2) analytical instruments available in the laboratory (3) identified or assumed environmental impact (4) previous detections in wastewater, surface water, and groundwater [14-15].

2. MATERIAL AND METHODS

2.1 Reagents and Chemicals

Ampicillin (AMP), Cefadroxil (CEF), Cloxacilin (CLO), Ciprofloxacin (CIP) and Ofloxacin (OFL) were used from Oxigen Analytical Laboratories Baddi, Distt. Solan H.P., India. . Methanol (HPLC-grade), Phosphoric acid, Potassium dihydrogen orthophosphate (KH_2PO_4), Dipotassium hydrogen phosphate anhydrous (K_2HPO_4), Sodium chloride, Sodium hydroxide, Acetone were purchased from Merck Germany. Purified water (resistance, 18.2MΩ cm) was prepared by passing water through an Ultra- Q, waters. HPLC (Agilent) equipped with a VWD detector. All the other chemicals were of analytical grade unless otherwise stated. Standard stock solutions of individual antibiotics were kept dark in the freezer at -10 ^{O}C and were freshly prepared every three months.

Table 1: Physicochemical properties of the inspected antibiotic compounds

Compound	Formula	Molecular weight	Antibiotic Class
CIP	$C_{17}H_{18}FN_3O_3$	331.3	fluoroquinolones
OFL	$C_{18}H_{20}FN_3O_4$	361.3	fluoroquinolones
AMP	$C_{16}H_{19}N_3O_4S$	349.4	β- lactam
CLO	$C_{19}H_{18}ClN_3O_5S$	435.8	β- lactam
CEF	$C_{16}H_{17}N_3O_5S$	363.3	Cephalosporins

2.2 Equipment

The HPLC performs with the system, Agilent 1260 Infinity Series with quaternary pump equipped with a detector, column component, sampler, quaternary pump and trigger. The chromatographic column was C18 ODS (250 x 4.6 mm, 5 µm Agilent Technologies, USA). Residual quantification of antibiotics was performed on the HPLC system. The liquid chromatograph is prepared with the particular wavelength of particular antibiotic and a 4.6 mm x 25 cm Intersil column that contains packing C18. A gradient program is used to with the mobile phase combining solvent A (Monobasic potassium phosphate at pH 5) and solvent B (methanol). The flow rate is about 1.0 ml / min and the injection volume was 100µl. Chromatograph the standard preparation as directed under the procedure and records the peak response under procedure.

2.3 Chromatographic conditions

A triple mobile phase with a gradient elution was used. Solvent A was Ultra-Q water (HPLC grade), solvent B was ACN (HPLC grade) and solvent C was MeOH. Set the flow rate at 0.5 ml /min, using the following composition for gradient HPLC Channel A (25%), B (25%), C (25%), D (25%). The flow rate was 1.0 ml/min and the Injection quantity was 20µL with column temperature was ambient (About 25^{0}C). The detector was used as UV- Detector at 273 nm.

2.4 Sampling and extraction of antibiotics from hospital wastewater samples

The first sampling station was Maharao Bhim Singh Hospital (MBS Hospital), the second sampling station was Jay Kay Lone Hospital (JK Lone Hospital) and the third sampling station

was New Medical College Hospital (NMC Hospital). Total nine samples were taken from these three hospitals. Out of these nine samples, three samples from municipal supply water, three samples from the surgical ward sewage tank and three samples were taken from sewage treatment plant tank. All samples were collecting during 10.00 AM to 12.00 PM. The liquid samples were collected in narrow mouth high density polyethylene (hdpe) amber reagent bottles with 500 ml capacity. The containers were pre-washed with distilled water several times and dried thoroughly before use. The surgical ward sewage tank was in depth (~3-4feet) and the STP tank were deep almost ~8-10 feet. After collecting the sample bottle was tightly closed & kept on -10^{o}C with dry ice so that the sample may not be degraded by the sunlight or UV rays until analysis. The containers in all cases were filled as much as possible and tightly stoppered to avoid contact with air or to prevent agitation during transport. All the samples were analyzed in Oxigen Analytical Laboratories, Baddi, Distt. Solan, HP, India.

2.5 Standard preparation

Weighed accurately 50 mg of the following antibiotics WS into a 50 ml volumetric flask. Added sufficient amount mobile phase, sonicate to dissolve, cool and dilute up to the mark with the mobile phase. Further, dilute 1 ml to 100 ml with the mobile phase.

2.6 Sample preparation

The raw sewage sample was filtered through a 0.45µm of membrane filter (Millipore) and adjusts the pH 8 using 2% sodium hydroxide solution. C18 cartridges, solid phase extraction columns were conditioned with 10mL of MeOH, 10mL of Ultra –Q water, 5mL of NaCl, 2% and 5mL of a 0.1M phosphate buffer solution (pH 8.0) to each sample (200-500 ml of the filtered sewage water). Thereafter the sample was passed through the SPE columns at a flow rate of approximately 2ml/min. The sorbent was washed with 5ml water and the antibiotics were eluted with 2×1ml of methanol/water (60:40 V/V) after a 5 min drying step with air, antibiotics were filtered through 0.22µm nylon syringe filter then dilutes 1ml to 200 ml with the mobile phase. Separately inject equal volume (100µL) of the blank, standard preparation and sample preparation into the chromatograph. Record the chromatograph and measure the responses for the major peaks.

2.7 Ethical approval

Approval for the study was obtained from the ethical committee of the New Medical College Hospital, Kota.

3. RESULTS AND DISCUSSION

In this study, five antibiotics were selected and in order to attain the separation a combination of methanol and 0.1M monobasic potassium phosphate was used as mobile phase. The samples were injected into HPLC with regard to the separation among the five target antibiotics and the sharpness of the peaks obtained upon injection of equal amounts. The complete separation of CIP, OFL, AMP, CLO and CEF and their peaks were obtained by a C-18 column (4.6 mm x 25 cm) and

a methanol and 0.1 M monobasic potassium phosphate was used as the mobile phase with a flow rate of 1.0 ml / min and the injection volume was 100µl.

3.1 Sample Analysis

Nine samples of wastewater from three sampling sites of three different hospitals were analysed for determination of residual levels of selected antibiotics. It was observed that in all three hospitals the concentration of CEF was much higher as compared to other antibiotics and the concentration of CLO was very low as compared to other antibiotics.

Table 2: Antibiotic concentration (in mg/L) in hospitals wastewater samples.

Antibiotics* (→) **Sample Sites (↓)**	**AMP**	**CLO**	**OFL**	**CEF**	**CIP**
Hospital 1 **MBS Hospital**	0.9726	0.2562	0.1231	2.7261	1.1586
Hospital 2 **JK Lone Hospital**	0.7784	0.1723	0.1704	1.7666	1.0645
Hospital 3 **NMC Hospital**	0.7134	0.1076	0.2928	1.9836	0.471
Mean Range	0.83	0.18	0.20	2.16	0.90

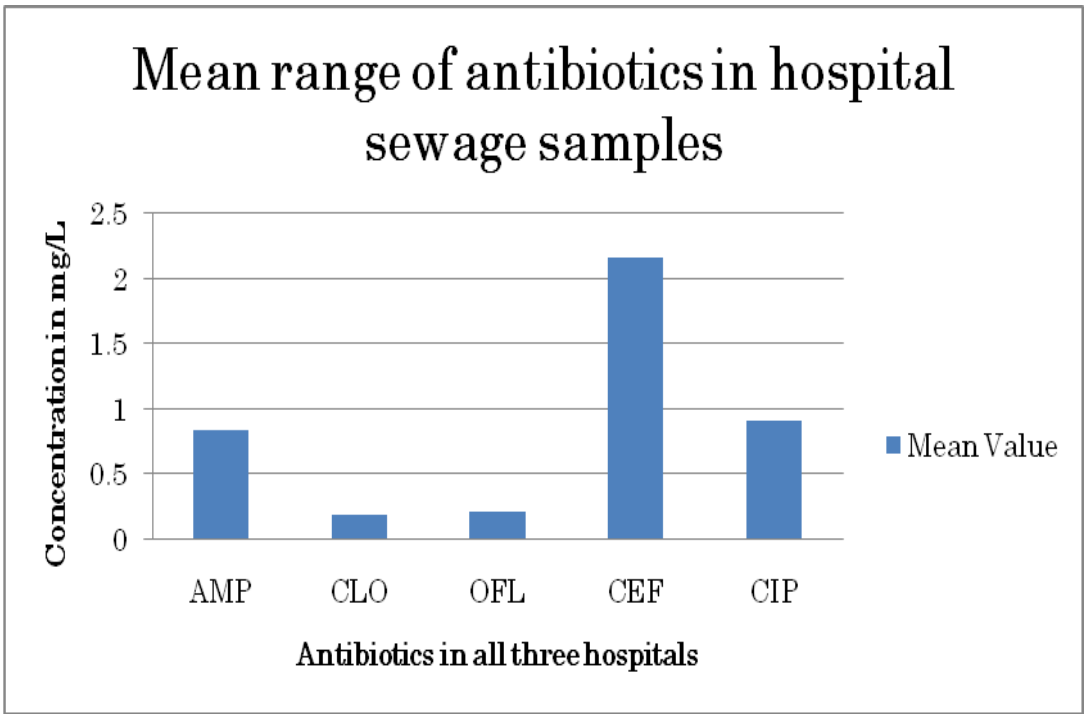

Figure 1: Graphical representation of mean concentrations of antibiotics

4. CONCLUSION

An SPE-HPLC method was used for the identification of five antibiotics in sewage water in Kota. The water samples were collected from three sites of each three hospitals. The present sewage treatment plant is not sufficient to dispose of antibiotics in wastewater. Lack of awareness, appropriate policy and laws, and apathy are responsible for improper management of medical waste in Kota City. The process of collection, segregation and disposal of medical waste do not perform according to recommended standards, and concerned people are exposed to the danger of such wastes. This may cause the development of bacterial resistance. Presence of stagnant water, the absence of scientific drainage, improper water-management, soil, etc. have to be thoroughly analyzed to evaluate the role of each of these factors in the bacterial resistance. The concentration of antibiotics was calculated in mg/L. The results of this study showed that CEF was detected in much high concentration 2.16 mg/L in sewage water in all three hospitals as compared to other antibiotics. The range of antibiotics concentration were CEF (2.16)>CIP (0.90)>AMP(0.83)>OFL(0.20)>CLO(0.18). The presence of antibiotics in sewage water may lead to potential emergence of resistant bacteria that should be considered in future studies. Finally, the implications of my findings may not be straightforward in relation to public health; nevertheless, my study highlights the need for more extensive investigation on the occurrence of antimicrobial compounds and bacterial resistance to them also in surface waters in Kota region.

5. ACKNOWLEDGEMENT

I wish to express my deepest gratitude to Dr. Pradeep Parasher, Associate Professor in Chemistry, Government College, Jhalawar, Rajasthan, for his valuable guidance, scholarly inputs and consistent encouragement I received throughout the research work. I owe my special thanks to Dr. Girish Verma, Principal, Government Medical College, Kota, for providing necessary facilities and gave me untiring help during my research. I am highly thankful to Dr. Chandra Shekhar Sushil, Head, Department of Psychiatry, New Medical College Hospital, Kota and Dr. Naveen Saxena, Assistant Professor, Department of Microbiology, Government Medical College, Kota, for their indispensable help during collection of the samples from the hospitals.

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