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Occurrence and Distribution of Antibiotic Substance in Waste Water from Hospital Effluent

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Abstract - Contamination levels of seven antibiotics, namely Ampicillin (AMP), Cifadroxil (CIF), Cefpodoxime (CEFPO), Cloxacilin (CLO), Ciprofloxacin (CIP), Ofloxacin (OFL) and Tinidazole (TIN) were identified in samples of wastewater of the three hospitals in Kota. HPLC with a VWD detector, C-18 column, and solid-phase cartridges were used to analyze antibiotic residues. In wastewater concentration level of residual antibiotics were found in the range 0.26 to 3.24 mg/L. Higher levels of residues were detected for CEF from Cephalosporins with residual levels of 3.24 mg/L. Other antibiotics were found in the level of AMP 1.24mg/L, CEFPO 0.28mg/L, CLO 0.31mg/L, OFL 0.28mg/L, CIP 1.35mg/L and TIN 0.26mg/L. OFL and CEFPO were found in minimum concentrations in all three hospitals wastewater. The contamination level was much higher in wastewater samples as it is the primary source of antibiotics entering the environment.

Keywords - Antibiotics, wastewater, HPLC, antibiotic resistance, environment.

I. INTRODUCTION

Antibiotics are used to treat and prevent epidemic diseases in humans, plants and animals. Antibiotics are naturally occurring, semi-synthesized or synthesized complexes with antimicrobial action. Many times the unused drugs or unmetabolized antibiotic substance is disposed into the sewage system. The presence of pharmaceuticals in the environment, gaining importance and it is a national and international issue nowadays. Although the concentration levels of pharmaceuticals in the environment may be very low, but they may be enough to have adverse effects on the environment and on humans. The long term exposure to the trace amounts and mixture of pharmaceuticals may have an effect on vulnerable population, including pregnant women, newborns and children [1, 2].If the drugs are not degraded or eliminated during sewage treatment plant, in soil or in other environmental compartments, they will reach surface water and ground water and potentially drinking water [3-7]. The wider use of pharmaceutical substance is scary for human health not only in terms of spreading resistant bacteria but also other environmental damage [8-10]. Fluoroquinolones like ciprofloxacin (CIP), ofloxacin (OFL), Penicillin like ampicillin (AMP), cloxacillin (CLO), Cephalosporins like cefadroxil (CEF), cefpodoxime proxetil (CEFPO), and Nitroimidazole like tinidazole (TIN) antibiotics are broad-spectrum antibiotics as they are effective against a wide variety of infectious diseases. These antibiotics are manufactured in generous quantities due to their extensive use in human and veterinary treatments. The use of therapeutic doses in animal to promote growth can lead to antibiotic resistance and food residues.

II. SOURCES OF ANTIBIOTICS IN THE ENVIRONMENT

Antibiotics have been polluting the environment since their introduction through human waste (medication, farming), animals, and the pharmaceutical industry. Along with antibiotic waste, resistant bacteria follow, thus introducing antibiotic-resistant bacteria into the environment. The elimination of unmetabolized antibiotics by humans and animals is one of the main causes of antibiotics in the environment [8]. Additional sources include the disposed of expired antibiotics and

leftovers from medical and industrial practices [11]. Residential and commercial facilities are identified suppliers of antibiotics to community wastewater [12]. The presence of stagnant water, the absence of scientific drainage, improper water-management, soil, etc. are also the reason of bacterial resistance. Frequent contact of the bacteria with even minor concentrations of antibiotics can cause the occurrence or persistence of resistant bacterial strains [13-17]. Many research proved that the concentration of the antibiotics in the environment showed hazardous properties [18, 19] and bad effects [20-22].

III. FATE AND OCCURRENCE OF ANTIBIOTICS IN THE ENVIRONMENT

Antibiotics have been found in a distinct environmental chamber all over the world, along with (i) sewage water [23-25] (ii) hospital wastewater [26, 27] (iii) ground and river water [28, 29] and (iv) soil and manure [30-32]. The lowest concentrations are generally found in the ground and river water while much higher concentration may be present in hospital effluents. The concentration of antibiotics decreases as they extend further into the environment due to some factors like: (i) phase distribution into STP (ii) their fate in STPs in terms of degradation and sorption (iii) dilution in recipient waters and (iv) a biotic and biotic degradation.

Fate of Antibiotics in the Environment of Kota

To my knowledge, this was the first study has been done in Kota region. There are three large government hospitals and approximately twenty private hospitals in the Kota city. Only the government hospitals are having sewage treatment plants, whereas these 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 wastewater. Usually wastewater from hospitals directly drain into the sewage system that in turn enters the canals used for crop irrigation. On the other hand, some amount of wastewater reaches ponds of stagnant water. These ponds are also a source of drinking water for animals. Aquatic life is affecting the most of this habit. So the most immediate need is to develop an easy, economic, reproducible, precise, and accurate method of quantification of residual levels of antibiotics in such samples. Ciprofloxacin (CIP) and ofloxacin (OFL) from fluoroquinolones, ampicillin (AMP) and cloxacillin (CLO) from penicillin, cefadroxil (CEF), cefpodoxime proxetil (CEFPO) from Cephalosporins, and tinidazole (TIN) antibiotics were selected for this study. The selection of antibiotics was based on five aspects:1) frequency of use 2) analytical instruments available in the laboratory 3) identified or assumed environmental impact 4) presence in aqueous environments and 5) previous detections in wastewater, surface water, and groundwater [33, 34].

IV. OBJECTIVE OF THIS STUDY

The aims of the studies underlying this thesis were to: (i) To develop a method for determining a wide range of antibiotics in environmental samples (ii) investigate the occurrence and to some extent the fate of antibiotics in the Indian environment, especially via STP and untreated municipal wastewater samples (iii) assess the potential antibiotic contaminants in wastewater.

V. EXPERIMENTAL

As for most trace substances, appropriate methods had to be developed and validated in order to measure the low quantities of antibiotic substances present in environmental samples accurately and precisely. In addition, the methods used had to be time- and cost-effective and to consume low amounts of material and solvents.

VI. CHEMICALS AND REAGENTS

Reference standards used for HPLC analysis of AMP, CLO, CIP, CEF, CEFPO, TIN and 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 an VWD detector. All the other chemicals were of analytical grade unless otherwise stated.

VII. EQUIPMENTS USED

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.

VIII. SAMPLING SITES

Three sites (A, B and C as shown in Fig. 1) were selected for the collection of aqueous wastewater samples. These three sites were different from each other. The first sampling site is a Maharao Bhim Singh Hospital (MBS Hospital), the second sampling site is Jay Kay Lone Hospital (JK Lone Hospital) and the third sampling site is 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. 500ml of wastewater sample from each sampling point was collected in narrow mouth high density polyethylene (hdpe) amber bottle. The depth of the surgical ward sewage tank was in ~3-4feet while the sewage treatment plant tank was almost ~8-10 feet. The sewage sample was collected through the cleaning and up keeping staff of the hospital. The sample from the tank was taken with the help of a bucket and after removing solid matters the sample poured into the amber bottle. After collecting the sample, the bottle was tightly closed & kept immediately into the dry ice box so that the sample is maintained -10^{o}C temperature and may not be degraded by the sunlight or UV rays. Necessary precautions were taken in collecting samples from hospital sewage tanks from a well mixed zone avoiding floating materials. All nine samples were collected during 12.00 to 2.00 PM. All samples were taken between 18th April to 23rd April 2017. Temperature and pH of each sample were noted at each site. Any kind of filtration or preservation (except for storing on dry ice) was not done before transfer to the laboratory.

Fig. 1. (A) Municipal supply water, (B) Surgical ward sewage tank and (C) Sewage treatment plant tank

IX. SAMPLE PREPARATION

Wastewater samples collected from each site were treated for purification and each sample was passed through solid phase cartridges and eluted with methanol. Purified samples were analyzed quantitatively for CIP, CEF, OFL, CLO, AMP, CEFPO, and TIN. For CIP , OFL and TIN a filtered and degassed mixture of buffer (3.4ml of H_3PO_4 in 1000ml of water, Adjust pH 3.0 with Triethylamine) and Acetonitrile in the ratio 870:130 delivered with the flow rate is about 1.0 ml / min. For AMP and CLO a filtered and degassed mixture of buffer (5.44gm of KH_2PO_4 in 1000ml of water; adjust the pH 5.0 with KOH) and Acetonitrile in the ratio of 750:250 delivered

with the flow rate 1.0ml/min. For CEF a filtered and degassed mixture of buffer (6.8 gm of KH_2PO_4 in 1000ml of water, Adjust the pH 5.0 with H_3PO_4) and Acetonitrile in the ratio of 960:40 delivered.

X. QUANTITATIVE ANALYSIS

Treated samples were analyzed quantitatively using high performance liquid chromatography (HPLC). The chromatographic system was equipped with a column of 4.6 mm x 25cm dimensions. 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. The retention time for each analyte is mentioned in Table I.

XI. ETHICAL APPROVAL

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

XII. RESULTS AND DISCUSSIONS

In this study, seven 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 to HPLC with regard to the separation among the seven target antibiotics and the sharpness of the peaks obtained upon injection of equal amounts. The complete separation of CIP, OFL, AMP, CLO, CEF, CEFPO and TIN 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.

XIII. 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 the residual concentration of CEF was much higher in each of the hospital wastewater samples. The hospital 1 has the highest level of CEF (4.0892 mg/L), the concentration level of CEF was in hospital 2 (2.6498 mg/L) and the level of CEF was in hospital 3 (2.9754 mg/L). The highest level of CIP was found in hospital 1 (1.8304 mg/L) and in hospital 2 and 3 the level of CIP was (1.6493 and 0.7253 mg/L).The Tinidazole was found in least quantity. The lowest level of antibiotic of TIN (0.0200 mg/L) was found in hospital 2. Hospital 1 was found to be most contaminated by selecting antibiotics levels and hospital 3 the least. In wastewater sample contamination levels the order of hospitals were1>2>3.

XIV. CONCLUSION

Antibiotics are a major source of contamination that reduces immunity in people as well as animals. Humans take antibiotics directly or indirectly from food items, meat, milk, and vegetables, etc., and these are accumulated in the body. Combination of methanol and 0.1M monobasic potassium phosphate was used as mobile phase in this method is useful for determining residual levels of antibiotics in hospital wastewater. We have found that three selected sites were contaminated by these antibiotics. No antibiotics were detected in the samples of municipal water supply from each three of the hospitals [35]. The residual levels were found in the range 0.26 to 3.24 mg/L in wastewater. Higher levels of residues were detected for CEF from Cephalosporins [36] with residual levels of 3.24 mg/L and CIP was detected at the second highest concentration [35]. Another fluoroquinolone, OFL, was detected in the lowest concentrations. High values of concentration of CEF indicate the presence of comparatively large amount of antibiotic due to (i) high dose consumption and (ii) 60% of drug eliminate unchanged. Other antibiotics were found in the level of AMP 1.24mg/L, CEFPO 0.28mg/L, CLO 0.31mg/L, OFL 0.28mg/L, CIP 1.35mg/L and TIN 0.26mg/L. OFL and CEFPO were found in minimum concentrations in all three hospitals sewage water. These results demonstrated that Cefadroxil and Ciprofloxacin is widely used in Kota district.

XV. REFERENCES

[1] Abuin S, Codony R, Compa R, Granados M, Prat MD (2006). Analysis of macrolide antibiotics in river water

by solid phase extraction and liquid chromatography-mass spectrometry, *J Chrom*, 1114, 2006, 73-81.

[2] Seifrtova M, Novakova L, Lino C, Pena A, Solich P (2009). An overview of analytical methodologies for the determination of antibiotics in environmental waters, *Anal Chim Acta*, 649, 2009, 158-179.

[3] NAKATA H. , KANNAN K., JONES P.D., GIESY J.P (2005). Determination of fluoroquinolone antibiotics in wastewater effluents by liquid chromatography-mass spectrometry and fl uorescence detection. Chemosphere. 58, (6), 759, 2005.

[4] GOLET E.M ., ALDER A.C., GIGER W (2002). Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. Environmental Science & Technology. 36, (17), 3645, 2002.

[5] CHANG X., MEYER M.T., LIU X., ZHAO Q., CHEN H., CHEN J.A., QIU Z., YANG L., CAO J., SHU W (2010). Determination of antibiotics in sewage from hospitals, nursery and slaughter house, wastewater treatment plant and source water in Chongqing region of Three Gorge Reservoir in China. Environ Pollut. 158 (5), 1444, 2010.

[6] HU X., ZHO U Q., LUO Y (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut. 158 (9), 2992, 2010.

[7] SUN J., L I W., ZHENG P., ZHU J (2012). Toxicity Evaluation of Antibiotics in Piggery Waste water by Luminescent Bacteria. Polish Journal of Environmental Studies. 21 (3), 641, 2012.

[8] LIU J.L. WONG M.H (2013). Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ Int. 59, 208, 2013.

[9] MACEDO A.S ., FREITAS A.R., ABREU C., MACHADO E., PEIXE L., SOUSA J.C., NOVAIS C (2011). Characterization of antibiotic resistant enterococci isolated from untreated waters for human consumption in Portugal. Int J Food Microbiol. 145 (1), 315, 2011.

[10] WALCZAK M ., DONDERSKI W (2004). Antibiotic Sensitivity of Neustonic Bacteria in Lake Jeziorak Mały. Pol. J. Environ. Stud. 13 (4), 429, 2004

[11] GLASSMEYER S.T ., HINCHEY E.K., BOEHME S.E., DAUGHTON C.G., RUHOY I.S., CONERLY O., DANIELS R.L., LAUER L., MCCARTHY M., NETTESHEIM T.G., SYKES K., THOMPSON V.G (2009). Disposal practices for unwanted residential medications in the United States. Environ Int. 35 (3), 566, 2009.

[12] BROWN K.D (2006). Pharmaceutically active compounds in residential and hospital effluent, municipal wastewater, and the Rio Grande in Albuquerque, New Mexico. 2011.

[13] OBST U., SCHWAR TZ T., VOLKMANN, H (2006). Antibiotic resistant pathogenic bacteria and their resistance genes in bacterial biofi lms. The International journal of artifi cial organs. 29 (4), 384, 2006.

[14] HANSON M.L., KNA PP C.W., GRAHAM D.W (2006). Field assessment of oxytetracycline exposure to the freshwater macrophytes Egeria densa Planch. and Ceratophyllum demersum L. Environ Pollut. 141 (3), 434, 2006.

[15] SELVARATNAM S. K UNBERGER J.D (2004). Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge. Can J Microbiol. 50 (8), 653, 2004.

[16] BAQUERO F., MAR TINEZ J.L., CANTON R (2008). Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol. 19 (3), 260, 2008.

[17] ABDALLAH S.A (2005) Detection and Differentiation of Escherichia coli Populations from Human, Animal and Avian Feces, and Different Water Sources. Pol. J. Environ. Stud. 14 (5), 639, 2005.

[18] SENDZIK J., SHA KIBAEI M., SCHÄFER-KORTING M., and STAHLMANN, R (2005). Fluoroquinolones cause changes in extracellular matrix, signalling proteins, metalloproteinases and caspase-3 in cultured human tendon cells. Toxicology. 212 (1), 24, 2005.

[19] PARK S. CHOI K (2008). Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology. 17 (6), 526, 2008.

[20] POMATI F., CASTI GLIONI S., ZUCCATO E., FANELLI R., VIGETTI D., ROSSETTI C., CALAMARI D (2006). Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells. Environmental science & technology. 40 (7), 2442, 2006.

[21] FANG H., HAN Y. , YIN Y., PAN X., YU Y (2014). Variations in dissipation rate, microbial function and antibiotic resistance due to repeated introductions of manure containing sulfadiazine and chlortetracycline to soil. Chemosphere. 96, 51, 2014.

[22] MUDRYK Z.J (2002). Antibiotic Resistance among Bacteria Inhabiting Surface and Subsurface Water Layers in Estuarine Lake Gardno. Polish Journal of Environmental Studies. 11, (4), 401, 2002.

[23] Hirsch, R.; Ternes, T.; Haberer, K.; Kratz, K.-L (1999). Occurrence of antibiotics in the aquatic environment. The Science of the Total Environment, 1999, 225, 109-118.

[24] Golet, E. M.; Alder, A. C.; Hartmann, A.; Ternes, T. A.; Giger, W (2001). Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. Analytical Chemistry, 2001, 73, 3632-3638.

[25] McArdell, C. S.; Molnar, E.; Suter, M. J.-F.; Giger, W (2003). Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt valley watershed, Switzerland. Environmental Science and Technology, 2003, 37, 5479-5486.

[26] Hartmann, A.; Golet, E. M.; Gartiser, S.; Alder, A. C.; Koller, T.; Widmer, R. M (1999). Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in German hospital wastewater. Archives of Environmental Contamination and Toxicology, 1999, 36, 115-119.

[27] Lindberg, R.; Jarnheimer P.A.; Olsen, B.; Johansson, M.; Tysklind, M (2004). Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. Chemosphere, 2004, 57, 1479-1488.

[28] Sacher, F.; Lange, F. T.; Brauch, H.-J.; Blankenhorn, I. (2001). Pharmaceuticals in groundwaters Analytical methods and results of a monitoring program in Baden-Württemberg, Germany. Journal of Chromatography A, 2001, 938, 199-210.

[29] Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science and Technology, 2002, 36, 1202-1211.

[30] Haller, M. Y.; Müller, S. R.; McArdell, C. S.; Alder, A. C.; Suter, M. J.F (2002). Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. Journal of Chromatography A, 2002, 952, 111-120.

[31] Hamscher, G.; Sczesny, S.; Höper, H.; Nau, H (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 2002, 74, 15091518.

[32] Pfeifer, T.; Tuerk, J.; Bester, K.; Spiteller, M (2002). Determination of selected sulfonamide antibiotics and trimethoprim in manure by electrospray and atmospheric pressure chemical ionization tandem mass spectrometry. Rapid Communication in Mass Spectrometry, 2002, 16, 663-669.

[33] HUANG C.H., SE DLAK D.L (2001). Analysis of estrogenic hormones in municipal wastewater effl uent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. Environmental Toxicology and Chemistry. 20 (1), 133-, 2001.

[34] BARNES K.K., KOLP IN D.W., MEYER M.T., THURMAN E.M., FURLONG E.T., ZAUGG S.D., BARBER L.B. (2002). Water-quality data for pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000. 2002: US Department of the Interior, US Geological Survey.

[35] La Thi Quynh Lien, Nguyen Quynh Hoa, Nguyen Thi Kim Chuc, Nguyen Thi Minh Thoa, Ho Dang Phuc, Vishal Diwan, Nguyen Thanh Dat, Ashok J. Tamhankar, and Cecilia Stålsby Lundborg (2016). Antibiotics in Wastewater of a Rural and an Urban Hospital before and after Wastewater Treatment, and the Relationship with Antibiotic Use—A One Year Study from Vietnam. *International Journal of Environmental Research and Public Health*. 2016 Jun; 13(6): 588. doi: 10.3390/ijerph13060588. PMCID: PMC4924045.

[36] Lindberg R H, Olofsson U, Rendahl P, Johansson MI, Behavior of fluoroquinolones and trimethoprim **d**uring mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. *Environmental Science & Technology*. ISSN 0013-936X, E-ISSN 1520-5851, Vol. 40, **N**o 3, 1042-1048 p. 2006

Table I
Retention time (RT) of different antibiotics at λmax

Antibiotics	**RT**	**λmax**
Ampicillin	5.3	230
Cefpodoxime proxetil	10.5	317
Cloxacillin	8.0	210
Ofloxacin	7.6	270
Cefadroxil	2.9	226
Ciprofloxacin	8.9	320
Tinidazole	4.5	315

Table II
Average concentrations of antibiotics in three Hospital wastewater (in mg/L)

Antibiotics→ Hospitals↓	**AMP**	**CEFPO**	**CLO**	**OFL**	**CEF**	**CIP**	**TIN**
MBS Hospital (1)	1.4589	0.3206	0.5289	0.1846	4.0892	1.7379	0.5964
JK Lone Hospital (2)	1.1676	0.3276	0.2583	0.2556	2.6498	1.5969	0.0200
NMC Hospital (3)	1.07	0.1758	0.1364	0.3891	2.9754	0.7065	0.1409
Mean Range	1.2322	0.2747	0.3079	0.2765	3.2382	1.3471	0.2525