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Detection of antibiotics cephtazidime and cephradine in hospital effluents and soil of Peshawar valley, Khyber Pakhtunkhwa province of Pakistan

Akhtar Hassan[1*], Muhammad Suleman[1], Aqleem Abbas[2], Nadia Khan[1], Muhammad Ilyas[1], Maria Ikhlas[1], Asma Akbar[2]

[1]*Department of Agricultural Chemistry, The University of Agriculture, Peshawar, Pakistan*

[2]*Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan*

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Abstract

The present study was carried out to detect the prevalence of antibiotics in the hospital effluents and soil of Peshawar valley, Khyber Pakhtunkhwa province of Pakistan. Two antibiotics were detected in the waste effluents, soil and drinking water of three hospitals including Hayatabad medical complex (HMC), Khyber teaching hospital (KTH) and Lady reading hospitals (LRH). The antibiotics included cephtazidime, (CPZM) and cephradine (CPDN). Samples collected from the selected locations were extracted by QuEChERS method technique followed by reversed phase high performance liquid chromatography. The results showed that the two selected antibiotics were detected in all the samples of HMC except CPZM which was not detected in the drinking water of HMC; however the waste water samples of HMC contained CPZM (0.47 µg mL^{-1}) in highest concentration among all the samples. It was revealed that waste water and soil of KTH contained all the selected antibiotics in higher concentrations than other samples. The waste water samples of KTH contained CPDN (0.64 µg mL^{-1}) in much higher concentration than other samples of KTH. The samples collected from LRH waste water contained the two selected antibiotics in higher concentration compared to other sites of LRH. CPDN (0.46µg mL^{-1}) is in the higher amount in hospital waste water among all the LRH samples. The results revealed that the two selected antibiotics were present at relatively higher concentration in the waste water and soil samples than the other samples. Further studies based on the risk assessment required to investigate the impact of these contaminants on the soil microbial community, aquatic organisms and human beings.

* **Corresponding Author:** Akhtar Hassan ✉ akhtar_turi2000@yahoo.com

Introduction

Antibiotics are the compounds, generated from microbial community, animal or vegetative cells suppressing micro organism growth and development. Antibiotics are likely the most successful group of pharmaceuticals so far developed for improving human health. Furthermore, antibiotics have also been utilized for protecting and curing animals and plants infections as well as for improving growth in animal farming (McManus *et al.,* 2002). All such applications have made antibiotics to be excreted in higher concentrations in natural ecosystems. A very little is known on the overall impacts of antibiotics on the community dynamics of the microbiosphere (Sarmah *et al.,* 2006). However, the effect of antibiotics used for treating infections or for farming purposes in the selection of antibiotic-resistant microorganisms, which can affect the human health (Witte, 1998). As stated by World Health Organization (WHO, 2002) the increasing emergence of antibiotic resistance in human pathogens is a special concern, not only for treating infectious disease, but also for other pathologies in which antibiotic prophylaxis is needed for avoiding associated infections.

Antibiotics have been used worldwide in human and veterinary medicine for therapeutic (disease control), prophylactic (disease prevention) and sub-therapeutic (growth promotion) purposes, contributing significantly to the improvement of our quality of life. In fact they are used to avoid or cure microbial infections and to improve the quality of growth of animals in livestock production (Ding and He, 2010). Nevertheless, the knowledge of what happens with antibiotics after their use, once they are released into the environment, has been very limited until now. For this reason the European Union prescribes the practical use of antimicrobial agents in human medicine (The Council of the European Union, 2002). Many thousands of tons of antibiotics are produced every year. According to data provided by the European Federation of Animal Health (FEDESA, 2001), about 13,000 tons of antibiotics were utilized by the EU and Switzerland in 1999, of which 65% was used in human medicine field, 29% was used in the veterinary medication and 6% as growth regulators. Antibiotics used in veterinary medicines penetrate into the environment commonly via manure, waste lagoon water and sewage sludge systems in the fields as plant fertilizers (Schlusener *et al.,* 2003). The overflow of antibiotics from animal livestock operations and seepage from storage lagoons or tanks are likely to contribute to the release of these compounds into the environment as well (Hao *et al.,* 2006). Moreover another source of pollution is the improper disposal of pharmaceutical waste arising from industrial activity. These are point sources that can contribute to high antibiotic concentrations in the environment. The naturally occurring antibiotics in the soil from, amongst others, bacteria and fungi control the dynamics of bacterial community. In contrast to these, most of the compounds used nowadays are semi-synthetic or synthetic (Stevens and McKinley 1995). Antibiotics and antivirals produced for humans and animals are naturally detected in the environment in sub therapeutic levels. Antibiotics have been detected in the µg L-1 range in municipal sewage, the effluent of sewage treatment plants (STPs), surface water and groundwater (Golet *et al.,* 2001). These concentrations can promote bacterial resistance (Sacher *et al.,* 2001) and consequently cause particular concern. The structures and functions of microbial community can be altered when they are exposed to antibiotics (Ding and He, 2010). The reason is that although most antibiotics have broad spectrum activity yet they have specific effects on different classes of micro-organisms. The classes of such micro-organisms could be broad like fungi or bacteria (Mohamed *et al.,* 2005) or they may be of specific spectrum as single genus or species (Yang *et al.,* 2009). Consequently, the selective antibiotic effects change the relative large quantity of microbial species and subsequently contribute with the interactions among different species. Interestingly, the effects on the microbial community depend on the original soil characteristics (Čermák *et al.,* 2008), microbial classes and the dose of antibiotics administered (Zielezny *et al.,* 2006). Moreover, the

ratios of bacteria/fungi and Gram-positive/Gram-negative bacteria (G+/G–) are the two important indicators most commonly used to show changes in microbial biodiversity. The aim of this study was to detect the antibiotics cephtazidime andcephradine in hospital effluents and soil of Peshawar valley, Khyber Pakhtunkhwa Province of Pakistan.

Materials and methods

Chemical and reagents

Two antibiotics working standards of cephtazidime and cephradine were provided by the Toxicology laboratory, Agricultural Chemistry, The University of Agriculture, Peshawar. All the chemicals including ethyl acetate, acetonitrile and water were of HPLC grade and purchased from Daejung (Siheung City, Korea). Acetic acid, $MgSO_4$, NaCl and Na_2 SO_4 were purchased from Merck (Germany).

Sampling

Water and soil samples were collected from various hospitals i.e. Khyber Teaching Hospital (KTH) Peshawar, Lady Reading Hospital (LRH) Peshawar and Hayatabad Medical Complex (CMH) Peshawar. From each hospital, three types of water samples were collected, i.e. drinking water of hospital, hospital waste effluents and hospital effluents from side areas of the hospital and two types of soil samples were collected i.e. soil from inside the hospitals and soil in close vicinity (approx. 100 meter) to hospital waste effluents. All such samples were immediately transported to the laboratory and were refrigerated for further analysis.

QuEChERS method

10 ml from each water sample and 5 gram from each soil sample in a felcon tube were taken and put 10 ml of ethyl acetate solution having 0.1% acetic acid in each tube. After this, the felcon tube was run on vortex for 1 minute and then 4 grams dry $MgSO_4$ and 1 gram NaCl were added and again run it on vortex for 1 minute. Now the mixture was centrifuged at 5000 rpm for 5 minutes and after this step the upper layer was taken in a dispersive column. After this procedure empty F indarf tube was taken and put to it 0.15gm $MgSO_4$, 0.05gm Alumina and 0.005 activated charcoal. The upper layer (from the felcon tube) was transferred to the dispersive column and centrifuged it at 3000rpm for 3 mints. After this the upper clear layer was taken and washed it with dry Na_2SO4 to completely remove water traces and further impurities from the extract. The extract was collected in a vial and carried it to the rotary evaporator for dryness.

In the rotary evaporator, the ethyl acetate completely evaporated and only the extracted sample (antibiotics) remained in the rotary flask. In the rotary flask a few drops of acetonitrile were put. Such acetonitrile (having cephtazidime and cephradine) in the rotary flask were placed in another sterilized vial for further analysis on HPLC.

HPLC working operation

Analysis of antibiotic standards and all the water and soil samples were done by Shimadzu HPLC employing reversed phase method. Briefly, an isocratic pump and a UV detector were used for the analysis of selected pharmaceuticals and the samples. Chromatographic separation was achieved on Waters Xterra C_{18} column (150mm x 4.6mm x 5µm) using phosphate buffer (pH 5) and acetonitrile in the ratio of 20: 80 as mobile phases. The mobile phase flow rate was 0.8 mL min^{-1}. The UV range for all the standards and samples was adjusted in the range between 180 to 300 nm. Hao *et al.* (2007) also developed a method for the analysis of various broad spectrum antibiotics using a hypersil C18 column and a mobile phase consisting of sodium dihydrogen phosphate and acetonitrile in the ratio of 15:85. The mobile phase flow rate was kept 1 ml per min and the UV detection range was between 200 to 300 nm.

The on column concentration of each standard injected was 0.2 µg. Same conditions were kept for the samples analysis. The areas and retention times of all the standards and samples were used for the quantification purpose. The concentrations of the selected compounds in the samples were determined by response factor obtained as,

$$Response\ Factor = \frac{\text{Peak area of the standard}}{\text{Conc. of the standard}}$$

By knowing the Response Factors of standards, we can easily determine the quantities of interested pharmaceuticals in the samples with the following formula,

$$Conc. of\ compound\ in\ sample = \frac{\text{Peak area of the compound in sample}}{\text{Response Factor}}$$

The concentrations of the selected compounds in the samples were calculated from the response factor obtained from the analysis of standard compounds.

Statistical Analysis

All the experiments were carried out in triplicate and the result was presented as mean ± S.E in the results.

Results and discussions

During this research a high performance liquid chromatography (HPLC) method was optimized for the analysis of pharmaceuticals at trace levels in water and soil using reversed phase method with different combinations of mobile phases to achieve a method of choice for better chromatographic separation. Two types of antibiotics i.e. cephtazidime and cephradine were determined. HPLC parameters including mobile phase containing acetonitrile and potassium phosphate buffer of pH 5 in the ratio of 80:20 at flow rate of 0.8 mL/min, a UV detection range from 180 to 300 nm were used. The injection volume of 20 µL for both standards and samples was used. The retention time and UV spectrum was used for identification while peak area of the compound was used for quantization purpose.

Table 1. Antibiotic concentration (µg mL^{-1}) in the soil and water samples collected from HMC.

Sampling sites	CPZM	CPDN
Hospital waste water	0.47±0.016	0.39±0.013
Hospital soil	0.41±0.077	0.36±0.032
Side waste water	0.38±0.072	0.31±0.098
Side soil	0.25±0.006	0.16±0.003
Drinking water	ND*	0.14±0.043

ND*;Not detected, CPZM; Cephtazidime, CPDN; Cephradine.

Table 2. Antibiotic concentration (µg mL^{-1}) in the soil and water samples collected from KTH.

Sampling sites	CPZM	CPDN
Hospital waste water	0.021±0.049	0.64±0.055
Hospital soil	0.31±0.091	0.50±0.076
Side area waste water	ND*	0.24±0.012
Side area soil	ND*	ND*
Drinking water	ND*	ND*

ND*;Not detected, CPZM; Cephtazidime, CPDN; Cephradine.

Cephtazidime

When injecting a standard solution of cephtazidime of 0.2 µg mL^{-1}, a good peak was established on the computing screen, the UV spectrum for which was found at 190 nm. The compound was eluted at a retention time of 4.37 min. This peak proved supportive while finding the quantities of cephtazidime in different water and soil samples.

Cephradine

When a standard solution of cephradine of 0.2 µg mL^{-1} was injected, an obvious chromatogram of cephradine was obtained. The chromatogram also shows that cephradine eluted at a retention time of 6.10 min and indicated a high UV response at 238 nm. This chromatogram was used to find the

concentration of cephradine at trace levels in many water and soil samples selected from various kinds of hospitals.

Monitoring of selected compounds in soil and water samples

The peak areas of the standard solutions and their retention times were used to calculate the response factor to find out the concentration of these compounds in water and soil samples. These concentrations are given in the following tables.

Table 3. Antibiotic concentration (µg mL^{-1}) in the soil and water samples collected from LRH.

Sampling sites	CPZM	CPDN
Hospital waste water	0.33±0.003	0.46±0.067
Hospital soil	0.30±0.093	0.31±0.012
Side area waste water	0.30±0.032	0.24±0.033
Side area soil	ND*	ND*
Drinking water	ND*	ND*

ND*;Not detected, CPZM; Cephtazidime, CPDN; Cephradine.

Hayat Abad Medical Complex (Hmc)

The result shows that antibiotics were found in the samples collected from the HMC sites. All the sampling sites contain both of the antibiotics except drinking water of HMC which did not show any concentration of cephtazidime; however the quantities of cephtazidime were higher as compare to cephradine. The hospital waste water contains both of the antibiotics in slightly higher concentration than the other samples of HMC. In the waste water sample, Cephtazidime (0.47 µg mL^{-1}) was present at highest concentration among all the samples of HMC. The presence of both cephtazidime and cephradine in the soil samples in relatively high amount might be due to high amount in waste water. The higher amount of both compounds in the samples suggest that these compounds are either used in large amounts or they are stable than the other compounds. Sievers *et al.* (1999) also reported that cephtazidime and cephradine were monitored in the Rancho hospital water up to a limit of 0.40 µg mL^{-1} and 0.42 µg mL^{-1} respectively. The waste water and soil samples collected from the surroundings of the hospitals also contained the selected compounds but at low concentration. Interestingly, the drinking water contained no cephtazidine but cephradine in a relatively lower concentration.

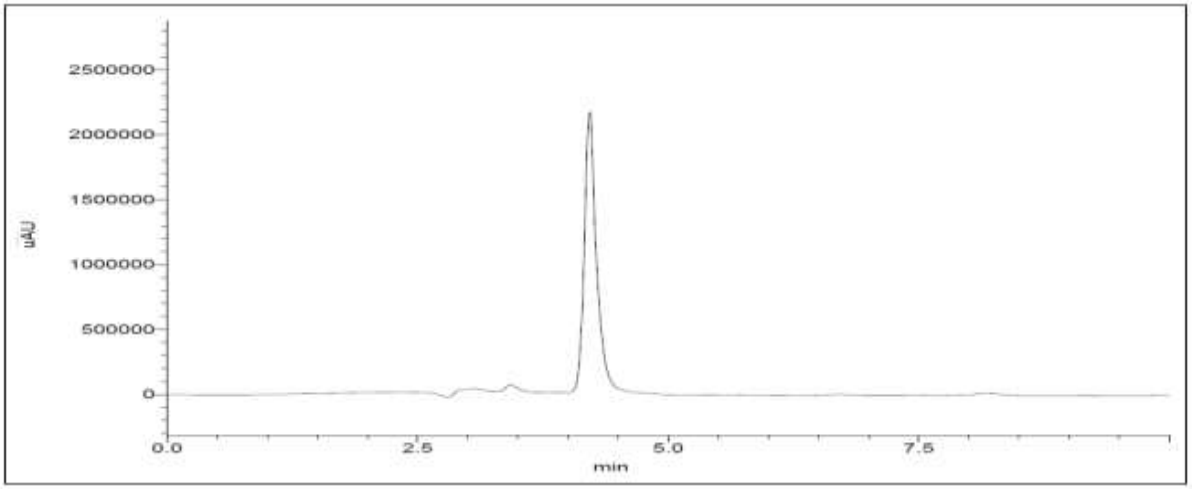

Fig. 1. HPLC Chromatogram of Cephtazidime obtained as a result of injection of standard Cephtazidime solution**.**

Khyber Teaching Hospital (Kth)

The above table (Table 2) shows the concentrations of the selected antibiotics in various water and soil samples collected from KTH sites. It was revealed that waste water and soil of KTH contains the two antibiotics in higher concentrations than the other samples. Gould and Richards, (2005) also investigated that cephradine was detected in soil with very small concentrations (0.30 µg mL^{-1}) due to its degradation and decomposition into their metabolites which may be dangerous and have an effect on the natural properties of soil, ground water, drinking water and human health. The waste water collected from the surrounding of KTH also contains cephradine (0.24 µg mL^{-1}) however no cephtazidime was detected in it. On the other hand none of the two compounds was detected in drinking water and side area soil samples. The presence these antibiotics in hospital waste water and hospital soil in relatively high quantity might be due to the presence of high amount in waste water and that might be deposited in the soil. Cephradine (0.64 µg mL^{-1}) was present in the highest level in hospital waste water.

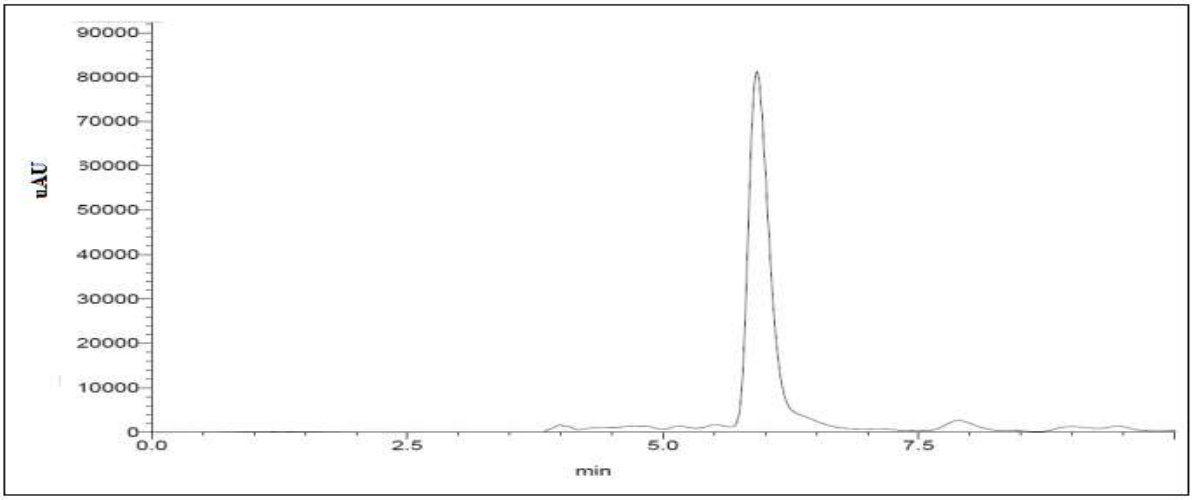

Fig. 2. HPLC Chromatogram of cephradine obtained as a result of injection of standard Cephradine solution.

Leady Reading Hospital (Lrh)

The results (Table 3) show that the samples collected from LRH waste water contained cephtazidime and cephradine in higher concentration as compared to other sites of LRH from which the samples were collected. Cephradine (0.46µg mL^{-1}) is in the highest amount in hospital waste water among all the LRH samples. The LRH hospital soil and LRH side area waste water also have the two antibiotics. It was also noted that the two antibiotics were absent in the drinking water of LRH and the soil samples collected from the surrounding of LRH. Heberer, (2002) analyzed surface water from the recipient stream and from two lakes that were contaminated by the nearby hospital effluents with high pressure liquid chromatography-mass spectrometry in Germany. These water samples were found to be contaminated with various types of pharmaceuticals such as ciprofloxacin, cetirizine and citalopram which were detected at more than 1 µg ml^{-1} while some others compounds were found relatively at a lower concentrations such as norfloxacin 0.53 µg ml^{-1}, cephtazidime 0.34 µg ml^{-1} and cephradine 0.27 µg ml^{-1} while the quantity of cephtazidime in LRH surroundings water was 0.30 µg ml^{-1} and cephradine was 0.24 µg ml^{-1}.

Conclusion

This was a preliminary study to find out the occurrence of selected antibiotics in the hospital effluents and surrounding soil. The results revealed that the selected compounds were present in the water as well as soil samples collected from the hospital and its close vicinity. Therefore we can conclude that; The waste water (hospital effluents) of all the three hospitals contain the selected antibiotics

relatively in high concentration as compared to other sampling sites i.e. hospital soil, side area waste water, side area soil and drinking water etc. However the drinking water of all the three hospitals contains either relatively low amount of the compounds or was not detected or absent. This also suggests that the hospital effluent has no impact on the hospital drinking water as the source of drinking water might be located far away from the point of hospital effluents. Moreover the reversed phase HPLC method was found to be simple, rapid and sensitive method for the determination of selected antibiotics in soil and water.

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