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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Role of Hospital Effluents in the Contribution of Antibiotics and Antibiotic Resistant Bacteria to the Aquatic Environment

Maqsood Ahmad[1], Amin U. Khan[1], Abdul Wahid[2], Zahid Ali Butt[3], Muhammad Farhan[1] and Farooq Ahmad[1]
[1]Sustainable Development Study Centre, G.C. University, Lahore, Pakistan
[2]Department of Environmental Sciences, Bahauddin Zakariya University, Multan, Pakistan
[3]Department of Botany, Abdul Wali Khan University, Mardan, Pakistan

Abstract: Water samples were collected from 18 sampling sites including the three hospitals their Municipal Wastewater (MWW) drains receiving the hospital sewage and from the river Ravi. The occurrence and fate of ofloxacin (OFLX) and resistance of *Vibrio cholerae* due to the presence of OFLX was studied. It was found that 16 out of 18 sites were contaminated by the antibiotic. Highest concentration was observed in hospital wastewater (HWW) ranged from 7.31 to 39.13 µg/L and amount of OFLX in the municipal wastewater before mixing the hospital sewage was 0.26 to 0.43 µg/L and after mixing it raised up to 0.54 to 1.29 µg/L. At the entering point of the MWW drains to the river, concentration 0.44 and 0.31 µg/L were observed in the two drains carrying the HWW to the river, respectively. Upstream concentration from the point of entering of the first and second drains was 0.14 and 0.22 µg/L while the downstream concentration was 0.18 and 0.37 µg/L, respectively. The antibiotic was not detected from both the samples collected from the entering point of the river to the city and before entering of the first MWW drain, whereas the after mixing the first drain the concentration 0.043 µg/L was observed. The bacteria isolated from HWW, MWW drains and the river Ravi were 83% 66% and 83% resistant to OFLX, respectively.

Key words: Ofloxacin, *Vibrio cholerae*, hospital wastewater, municipal wastewater

INTRODUCTION

During the last many years, production of pharmaceuticals rapidly increased and approximately 3000 compounds are in use as medicine and annual production reached up to hundreds of tons (Sim *et al*., 2011). Among the large number of pharmaceutical compounds antibiotics are special because of their wide use in humans and animals, multiple sources and ability to produce resistant bacteria in the environment (Karthikeyan and Meyer, 2006). The first antibiotic discovered by man was penicillin produced by fungi. Now both synthetic like sulfa group and semi-synthetic like cephalosporin group antibiotics are available (Kummerer, 2009). It was estimated that worldwide annual consumption of antibiotics is about 100,000 to 200,000 tons. Most of antibiotics are not completely metabolize in bodies of human and animals and about 25 to 75% of added antibiotics excrete into the environment in active form through urine and feces, so antibiotics used by humans can be found at various concentrations in hospital effluents, municipal wastewater and discharge of wastewater treatment plants. (Zheng *et al*., 2011; Brown *et al*., 2006 and Chee *et al*., 2001). Antibiotics used by animals, reached to soils by grazing animals or manure used as fertilizer and the antibiotics can be reached to rivers by rainwater from the top soil (Zarfl *et al*. 2009). Antibiotics reported in groundwater (Sacher *et al*., 2001), soil (Jacobsen *et al*., 2004) sediments (Kim and Carlson, 2006) and even in drinking water (Watkinson *et al*., 2009).

In natural environment some antibiotics degrade very fast while some are can persistent for a long time (Jiang *et al*., 2010). The removal processes of antibiotics from the environment include thermolysis, photolysis, oxidation, hydrolysis and biodegradation (Kummerer, 2009). The release of antibiotics into the aquatic environment is responsible to produce single and multi drug resistant strains that belong to pathogenic bacteria (Servais and Passerat, 2009). The resistant bacteria can be found from lakes (Edge and Hill, 2005), rivers (Ash *et al*., 2002; Watkinson *et al*., 2007), estuaries (Parveen *et al*., 1997) and in costal wastewaters (Erdem *et al*., 2007). In most of aquatic environments the concentrations of antibiotics are very low but they have tendency to convert nonpathogenic bacteria to pathogenic bacteria by increasing the resistance against antibiotics (Kim *et al*., 2007, 2010). The continuous exposure even to low concentrations of antibiotics can produce resistant strains of bacteria in environment (Herwig *et al*., 1997; Kemper, 2008; Duong *et al*., 2008; Yu *et al*., 2009). During the recent years antibiotics became to know as high risk pollutants because of their toxic effects on bacteria and algae at very low concentrations and also due to their capacity to create resistant amongst natural bacterial populations (Watkinson *et al*., 2009).

Corresponding Author: Maqsood Ahmad, Sustainable Development Study Centre, G.C. University, Lahore, Pakistan

Present study was carried out to address two main issues: To investigate the presence of an important fluoroquinolone antibacterial agent (OFLX) from hospital effluents and also from municipal wastewater drains of Lahore receiving the hospitals wastewater.

To explore the persistence of antibiotic-resistance-bacteria from wastewaters of selected sites.

To create awareness about the vital significance of aquatic environment.

MATERIALS AND METHODS

Hospital wastewater samples were collected from three hospitals of Lahore i.e., Mayo, Services and Jinnah. Composite samples were collected from the hospital wastewater during different timings (7-11, 11-15, 15-19 and 19-23 hrs.) to study the temporal variation in the concentration of the targeted antibiotic. Composite samples were also collected from municipal wastewater drains that are receiving the wastewater from the hospitals under study. Quantity of each sample was 4 L and were collected in amber glass bottles and transferred to laboratory in ice box and preserved with Na_2EDTA at 4°C (Chang *et al.*, 2010).

The raw wastewater samples were filtered through 0.45 µm membrane filter and H_2SO_4 was added to attain 3 pH. (Lindberg *et al.*, 2004). Extraction of the antibiotic was carried out by Solid Phase Extraction (SPE); the cartridges were first rinsed with 2 ml of methanol followed by 2 ml of water. The required compounds were eluted from the cartridges by using 2 ml of methanol. The extracted samples were analyzed by high performance liquid chromatography (HPLC) equipped with UV-visible and photodiode array detector. The analytical column RP-18e 250mm×4.6mm, 5 µm manufactured by Merck was used at room temperature for analysis. Five ml methanol-water (50:50), pH 3.0 used as conditioning solvent, 5 ml water (pH 3.0) as washing solvent and 5 ml triethylamine 5% in methanol was used as elution solvent (Brown *et al.*, 2006; Seifrtova *et al.*, 2009).

Identification and isolation of *Vibrio cholerae* was carried out by selective growth media Oxoid TCBS, by incubating at 35°C for 24 hours. Yellow flat 2-3mm diameter colonies were selected as *Vibrio cholerae*. The isolated *Vibrio cholerae* were subcultured on the nutrient agar and then kept at 4°C for further examination (Koczura *et al.*, 2012; Luczkiewicz *et al.*, 2010).

To observe the resistance level of *Vibrio cholerae* different concentrations of ofloxacin i.e., 0, 0.015, 0.06, 0.2, 1, 10, 30, 50, 100 µg/ml were prepared by using Oxoid Iso-sensitest broth, the pathogens were incubated at 37°C for 24 hours and monitor the growth of the bacteria (as turbidity) by measuring optical density at 600 nm. Duplicate samples of each antibiotic concentration were grown (Baines, 1993; Roychoudhury *et al.*, 2001, Theron *et al.*, 2000).

RESULTS AND DISCUSSION

The concentration of OFLX was measured in untreated sewage of the three hospitals ranged from 35.62 to 7.31 µg/L (Table 1). The results are comparable to the findings of Verlicchi *et al.* (2012), who reported concentration of OFLX ranged from 3.7 to 31 µg/L in hospital sewage. Comparable value of the targeted compound was also recorded as 13.6 µg/L by Lin *et al.* (2008). While the values are higher than 4.2 and 7.6 µg/L of OFLX in hospital sewage as reported by Chang *et al.* (2010) and Lindberg *et al.* (2004), respectively. Temporal variation in concentration of OFLX was observed in all the samples of each hospital collected from 7:00 to 23:00 hrs (Table 1). Lindberg *et al.* (2004) investigated ofloxacin, ciprofloxacin, sulfamethoxazol, trimethoprim, metronidazole from hospital wastewater and reported temporal variation in concentrations of all the antibiotics under study. Variation in the concentration of antibiotics were observed 8:00 am to 11:00 pm, these variation are due to different factors like time of consumption and excretion through urine and feces (Duong *et al.*, 2008). Verlicchi *et al.*, 2010 studied different pharmaceutical compounds in hospital wastewater and reported variation between 12:00 AM to 10:00 PM.

Amount of OFLX in the three municipal wastewater drains before mixing the respective hospital wastewater ranged from 0.43 to 0.3 µg/L and after mixing concentration increased up to 1.29 to 0.54 µg/L (Table 1). Concentration in Shadman drain carrying the wastewater of services and Mayo hospitals and Satokatla drain carrying wastewater of Jinnah hospital for river Ravi were 0.44 to 0.31 µg/L just before entering the river (Table 2). A similar concentration range for OFLX in treatment plant has been recorded as 0.44-3.1 µg/L (Gao *et al.*, 2012). Peng *et al.*, 2006 investigate sulphadiazine, sulfamethoxazole, ofloxacin and chloramphenicol in the effluent of sewage treatment plant and found the concentration of ofloxacin ranged from 3.52 to 5.56 µg/L. Deblonde *et al.*, 2011 reported 2.275 µg/L ofloxacin in effluents of wastewater treatment plant. Lee *et al.* (2007) reported .034 to 0.251 µg/L in the wastewater is also comparable to the values of the present study.

Concentration of ofloxacin in the river before mixing the Shadman and Satokatla drain were 0.14 and 0.22 µg/L and after mixing range was 0.18 and 0.37 µg/L, respectively (Table 2). Upstream and downstream concentrations of ofloxacin at the point where first wastewater drain entered to the river were also examined. Ofloxacin was not detected upstream whereas downstream concentration was 0.043 µg/L (Table 3). The presence of ofloxacin in different rivers reported by number of studies (Dinh *et al.*, 2011; Tamtam *et al.*, 2008; Jiang *et al.*, 2011; Xu *et al.*, 2007; Yiruhan *et al.*, 2010; Zhang *et al.*, 2012; Zhou *et al.*,

Table 1: Concentration of antibiotics in wastewater of the three hospitals

		Concentrations in (µg L^{-1})				Municipal wastewater drain	
		Time of sampling (hrs)				17 hrs composite sampling	
Antibiotic	Hospital	07:00 to 11:00	11:00 to 15:00	15:00 to 19:00	19:00 to 23:00	Before mixing	After mixing
	Mayo	22.19b±1.40	35.62b±0.82	18.41b±1.13	32.76b±0.19	0.26b±0.07	0.85±0.06
Ofloxacin	Services	27.32a±0.62	39.13a±1.05	20.33a±0.35	35.21a±0.81	0.43a±0.03	1.29±0.18
	Jinnah	9.23c±0.52	14.42c±0.11	7.31c±0.61	11.80c±0.94	0.33c±0.03	0.54±0.06

Treatment means followed by different letters in each column for Ofloxacin are significantly different at p = 0.05 according to Duncan's Multiple Range Test

Table 2: Concentration of the antibiotic in wastewater drains and river Ravi

		Concentrations (µg L^{-1})	
		River Ravi	
	Just before entering to the river	Upstream	Downstream
Shadman drain (Mayo and Services)	0.44±0.03	0.14±0.02	0.18±0.02
Satokatla drain (Jinnah)	0.31±0.06	0.22±0.03	0.37±0.06

Table 3: Concentration of the antibiotic before and after entering the first MWW drain

Concentrations of ofloxacin (µg L^{-1})		
Before entering Lahore	Before entering first WW drain	After mixing first drain
ND	ND	0.19±0.004

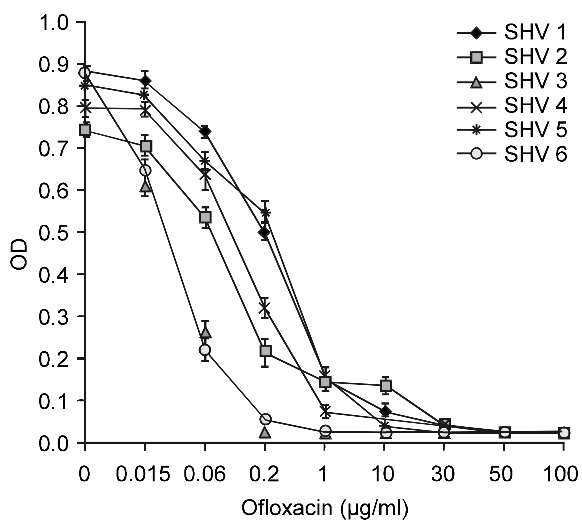

Fig. 1: Resistance of *Vibrio cholerae* collected from hospital wastewater

2011 and Zou *et al*., 2011) supporting the results of present study.

The *Vibrio cholerae* isolated from the hospital wastewater sample, 83% were found resistant for ofloxacin (Fig. 1). Isolates collected from municipal wastewater drain before mixing the hospital wastewater, 66% were resistant to the antimicrobial agent (Fig. 2). It is very much clear from the Fig. 1 and 2 that isolates from the hospital wastewater can

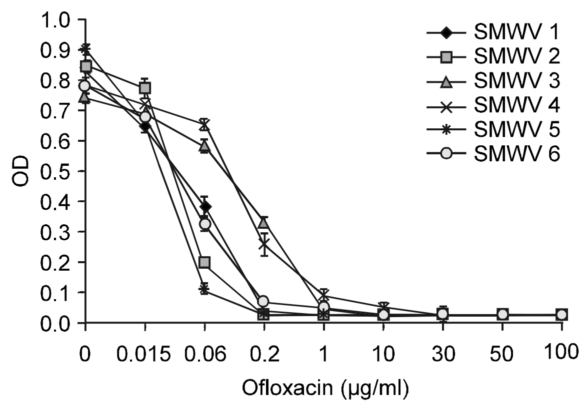

Fig. 2: Resistance of *Vibrio cholerae* isolated from MWW drain before mixing hospital wastewater

survive at higher concentrations as compare to the isolated of municipal wastewater drain.

The bacteria isolated from the river water sample collected from downstream after mixing of the Satokatla drain, the last drain entering in the river with municipal wastewater of Lahore were found 83% resistant (Fig. 3). Moore *et al*. (2010) reported resistance for bacterial isolates of river water for different antibiotics ranged from 21 to 92.9%. *E. coli* collected from Dongjiang river of China were found resistant up to 89.2% Su *et al*. (2012). Hoa *et al*. (2011) found that bacteria were resistant to sulfamethoxazole ranged from 2.14 to 94.44% and 0.01 to 38% bacteria were found resistant to erythromycin.

Dias *et al*. (2012) isolated 238 bacteria from bottled water of different brands and studied for antibiotic resistance. They reported that all the isolates were resistant to at least three antibiotics. Isolates from clinical wastewater showed high resistant to antibiotics than the wastewater isolates. About 50% clinical

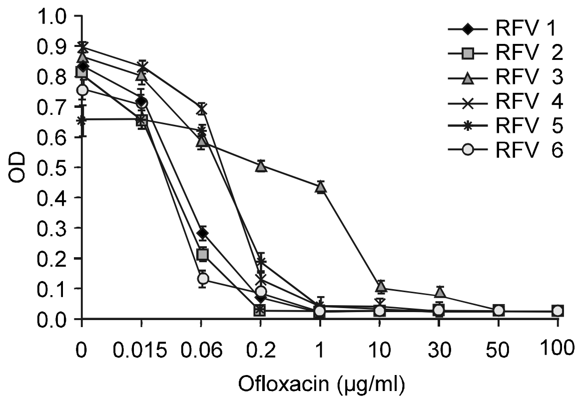

Fig. 3: Resistance of *Vibrio cholerae* collected from river Ravi after mixing Satokatla wastewater

isolates were resistant to antibiotics whereas the sewage isolates were found resistant upto 38%. So it is clear that the sewage which receiving untreated hospital wastewater is found to be a major source of multidrug resistance bacteria to environment (Santoro *et al*., 2012).

Conclusions: The results illustrated that the concentration of OFLX varied from time to time in the wastewater of the hospitals. Hospital wastewater was found main contributor of the antibiotic to the MWW drains. The river Ravi was not contaminated by the antibiotic before entering the first MWW drain. Therefore, the amount of antibiotic found in the river was only due to the MWW drains of Lahore entering the river from different points. The wastewater of the hospitals carrying more resistant *Vibrio cholerae* as compare to MWW drains. The river water is using for irrigation and livestock; the use can cause serious health problems to both humans and livestock because of the resistant bacteria. Hospitals must be equipped with wastewater treatment plants and municipals wastewater of Lahore must be treated before entering into the river.

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REFERENCES

Ash, R.J., B. Mauck and M. Morgan, 2002. Antibiotic resistance of gram-negative bacteria in rivers, United States. Emerging Infectious Disease, 8: 713-726.

Baines, W., 1993. Detecting antibiotics and biocides in water using a bacterial bioassay. Genet. Eng. Biotechnol., 13: 149-159.

Brown, K.D., J. Kulis, B. Thomson, H. Timothy, Chapman and B.D., Mawhinney, 2006. Occurrence of antibiotics in hospital, residential and dairy effluent, municipal wastewater and the Rio Grande in New Mexico. Sci. Total Environ., 366: 772-783.

Chang, X., M.T. Meyer, X. Liu, Q. Zhao, H. Chen, J. Chen, Z. Qiu, L. Yang, J. Cao and W. Shu, 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: 1444-1450.

Chee, S.J.C., R.I. Aminov, I.J. Krapac, J.N. Garrigues and R.I. Mackie, 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Applied Environ. Microbiol., 67: 1494-1502.

Deblonde, T., C.C. Leguille and P. Hartemann, 2011. Emerging pollutants in wastewater: A review of the literature. Int. J. Hygiene Environ. Health, 214: 442-448.

Dias, M.F.F., I.V. Moreira and C.M. Manaia, 2012. Bottled mineral water as a potential source of antibiotic resistant bacteria. Water Res., 46: 3612-3622.

Dinh, Q.T, F. Alliot, E.M. Guigon, J. Eurin, M. Chevreuil and P. Labadie, 2011. Measurement of trace levels of antibiotics in river water using on-line enrichment and triple-quadrupole LC-MS/MS. Talanta, 85: 1238-1245.

Duong, H.A., N.H. Pham, H.T. Nguyen, T.T. Hoang, H.V. Pham, V.C. Pham, M. Berg, W. Giger and A.C. Alder, 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital waste waters in Hanoi, Vietnam. Chemosphere, 72: 968-973.

Edge, T.A and S. Hill, 2005. Occurrence of antibiotic resistance in Escherichia coli from surface waters and fecal pollution sources near Hamilton, Ontario. Can. J. Microbiol., 51: 501-505.

Erdem, A.K., E.O. Arslan, N.O.S. Yurudu, Z. Zeybek, N. Dogruoz and A. Cotuk, 2007. Isolation and identification of enterococci from seawater samples: assessment of their resistance to antibiotics and heavy metals. Environ. Monit. Assess., 125: 219-228.

Gao, L., Y. Shi, W. Li, H. Niu, J. Liu and Y. Cai, 2012. Occurrence of antibiotics in eight sewage treatment plants in Beijing, China. Chemosphere, 86: 665-671.

Herwig, R.P., J.P. Gray and D.P. Weston, 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. Aquaculture, 149: 263-283.

Hoa, P.T. P., S. Managaki, N. Nakada, H. Takada, A. Shimizu, D. H. Anh, P. H. Viet and S. Suzuki, 2011. Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. Sci. Total Environ., 409: 2894-2901.

Jacobsen, A.M., B.H. Sorensen, F. Ingerslev and S.H. Hansen, 2004. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr, 1038: 157-170.

Jiang, L., X. Hu, D. Yin, H. Zhang and Z. Yu, 2011. Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China. Chemosphere, 82: 822-828.

Jiang, M.X., L.H. Wang and R. Ji, 2010. Biotic and abiotic degradation of four cephalosporin antibiotics in a lake surface water and sediment. Chemosphere, 80: 1399-1405.

Karthikeyan, K.G. and M.T. Meyer, 2006. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Science of the Total Environment, 361: 196-207.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators, 8: 1-13.

Kim, S., J.N. Jensen, D.S. Aga and A.S. Weber, 2007. Tetracycline as a selector for resistant bacteria in activated sludge. Chemosphere, 66: 1643-1651.

Kim, S., H. Park and K. Chandran, 2010. Propensity of activated sludge to amplify or attenuate tetracycline resistance genes and tetracycline resistant bacteria: A mathematical modeling approach. Chemosphere, 78: 1071-1077.

Kim, S.C. and K. Carlson, 2006. Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. Water Res., 40: 2549-2560.

Koczura, R., J. Mokracka, L. Jablonska, E. Gozdecka, M. Kubek and A. Kaznowski, 2012. Antimicrobial resistance of integron-harboring *Escherichia coli* isolates from clinical samples, wastewater treatment plant and river water. Sci. Total Environ., 414: 680-685.

Kummerer, K., 2009. Antibiotics in the aquatic environment. A review Part I. Chemosphere, 75: 417-434.

Lee, H.B., T.E. Peart and M.L. Svoboda, 2007. Determination of ofloxacin, norfloxacin and ciprofloxacin in sewage by selective solid-phase extraction, liquid chromatography with fluorescence detection and liquid chromatography tandem mass spectrometry. J. Chromatograp. A, 1139: 45-52.

Lin, A.Y.C., T.H. Yu and C.F. Lin, 2008. Pharmaceutical contamination in residential, industrial and agricultural waste streams: Risk to aqueous environments in Taiwan. Chemosphere, 74: 131-141.

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

Luczkiewicz, A., K. Jankowska, S. F. Ksiazek and K.O. Neyman, 2010. Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Res., 44: 5089-5097.

Moore, J.E., P.J.A. Moore, B.C. Millar, C.E. Goldsmith, A. Loughrey, P.J. Rooney and J.R. Rao, 2010. The presence of antibiotic resistant bacteria along the River Lagan. Agric. Water Manage., 98: 217-221.

Parveen, S., R.L. Murphree, L. Edmiston, C.W. Kaspar, K.M. Portier and M.L. Tamplin, 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of Escherichia coli in Apalachicola bay. Applied Environ. Microbiol., 63: 2607-2612.

Peng, X., Z. Wang, W. Kuang, J. Tan and K. Li, 2006. A preliminary study on the occurrence and behavior of sulfonamides, ofloxacin and chloramphenicol antimicrobials in wastewaters of two sewage treatment plants in Guangzhou, China. Sci. Total Environ., 371: 314-322.

Roychoudhury, S., C.E. Catrenich, E.j. Mcintosh, H.D. Mckeever, K.M. Makin, P.M. Koenigs and B. Ledoussal, 2001. Quinolone resistance in staphylococci: activities of new nonfluorinated quinolones against molecular targets in whole cells and clinical isolates. Antimicrobial Agents Chemotherapy, 45: 1115-1120.

Sacher, F., F.T. Lange, H.J. Brauch and I. Blankenhorn, 2001. Pharmaceuticals in groundwater. Analytical methods and results of a monitoring program in Baden-Wurttemberg, Germany. J. Chromatography A, 938: 199-210.

Santoro, D.O., C.M.C.A. Romao and M.M. Clementino, 2012. Decreased aztreonam susceptibility among *Pseudomonas aeruginosa* isolates from hospital effluent treatment system and clinical samples. Int. J. Environ. Health Res., 1-11, iFirst article.

Seifrtova, M., L. Novakova, C. Lino, A. Pena and P. Solich, 2009. An overview of analytical methodologies for the determination of antibiotics in environmental waters. Anal. Chimica Acta., 649: 158-179.

Servais, P. and J. Passerat, 2009. Antimicrobial resistance of fecal bacteria in waters of the Seine river watershed (France). Sci. Total Environ., 408: 365-372.

Sim, W.J., J.W. Lee, E.S. Lee, S.K. Shin, S.R. Hwang and J.E. Oh, 2011. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. Chemosphere, 82: 179-186.

Su, H.C., G.G. Ying, R. Tao, R.Q. Zhang, J.L. Zhao and Y.S. Liu, 2012. Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in Escherichia coli isolated from Dongjiang River, South China. Environ. Pollut., 169: 42-49.

Tamtam, F., F. Mercier, B.L. Bot, J. Eurin, Q.T. Dinh, M. Clement and M. Chevreuil, 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci. Total Environ., 393: 84-95.

Theron, J., J. Cilliers, M.D. Preez, V.S. Brozel and S.N. Venter, 2000. Detection of toxigenic Vibrio cholerae from environmental samples by an enrichment broth cultivation-pit-stop semi-nested PCR procedure. J. Applied Microbiol., 89: 539-546.

Verlicchi, P.A. Galletti, M. Petrovic and D. Barcelo, 2010. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. J. Hydrol., 389: 416-428.

Verlicchi, P., M. Al Aukidy, A. Galletti, M. Petrovic and D. Barcelo, 2012. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Sci. Total Environ., 430: 109-118.

Watkinson, A.J., G.B. Micalizzi, G.M. Graham, J.B. Bates and S.D. Costanzo, 2007. Antibiotic-resistant Escherichia coli in wastewaters, surface waters and oysters from an urban riverine system. Applied Environ. Microbiol., 73: 5667-5670.

Watkinson, A.J., E.J. Murby, D.W. Kolpin and S.D. Costanzo, 2009. The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. Sci. Total Environ., 407: 2711-2723.

Xu, W., G. Zhang, S. Zou, X. Li and Y. Liu, 2007. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Environ. Pollut., 145: 672-679.

Yiruhan, Q., C. Wang, Y.W. Mo, P. Li, Y.P. Gao, Y. Tai, Z.L. Zhang, J.W. Ruan and Xu, 2010. Determinationof four fluoroquinolone antibiotics in tap water in Guangzhou and Macao. Environ. Pollut., 58: 2350-2358.

Yu, D.J., X.L. Yi, Y.F. Ma, B. Yin, H.L. Zhuo, J. Li and Y. Huang, 2009. Effects of administration mode of antibiotics on antibiotic resistance of Enterococcus faecalis in aquatic ecosystems. Chemosphere, 76: 915-920.

Zarfl, C., J. Klasmeier and M. Matthies, 2009. A conceptual model describing the fate of sulfadiazine and its metabolites observed in manure-amended soils. Chemosphere, 7: 720-726.

Zhang, R. G. Zhang, Q. Zheng J. Tang, Y. Chen, W. Xu, Y. Zou and X. Chen, 2012. Occurrence and risks of antibiotics in the Laizhou Bay, China: Impacts of river discharge. Ecotoxicol. Environ. Safety, 80: 208-215.

Zheng, S., X. Qiu, B. Chen, X. Yu, Z. Liu, G. Zhong, H. Li, M. Chen, G. Sun, H. Huang, W. Yu and D. Freestone, 2011. Antibiotics pollution in Jiulong River estuary: Source, distribution and bacterial resistance. Chemosphere, 84: 1677-1685.

Zhou, L.J., G.G. Ying, J.L. Zhao, J.F. Yang, L. Wang, B. Yang and S. Liu, 2011. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. Environ. Pollut., 159: 1877-1885.

Zou, S. W. Xu, R. Zhang, J. Tang, Y. Chen and G. Zhang, 2011. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities. Environ. Pollut., 159: 2913-2920.