INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 3, p. 232-239, 2017

RESEARCH PAPER OPEN ACCESS

Prevalence of antibiotics and antibiotic resistant bacteria in wastewaters - An impact of untreated municipal and hospital wastewater

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Key words: Hospital wastewater, Antibiotics, Resistant bacteria.

http://dx.doi.org/10.12692/ijb/11.3.232-239 Article published on September 30, 2017

Abstract

Present study investigated the prevalence of some antibiotics viz., ciprofloxacin (CIP), levofloxacin (LFO) and ofloxacin (OFL) in hospital wastewater and seasonal drain receiving untreated municipal wastewater from Sialkot city. Bacterial strains including *Escherichia coli* (*E. coli*) and *Salmonella typhimurium* (*S. typhi*) were isolated from the raw hospital wastewaters and from the drain to investigate antibiotic resistance level. Concentration of CIP, LFO and OFL in hospital wastewater was measured up to 189µg L^{-1}, 142µg $L^{-1}$and 116µg L^{-1} respectively. No antibiotic was detected from the drain water upstream to the first municipal wastewater entering from the city. From the water samples, collected from downstream to the city all the three antibiotics, for instance CIP (0.091µg L^{-1}), LFO (0.078µg L^{-1}) and OFL (0.073µg L^{-1}) were detected. Isolates of *E. coli* (n=20) collected from hospital wastewater were 90% resistant to CIP, 75% resistant to LFO and 60% resistant to OFL. While the isolates of *S. typhi* (n=20) were 95%, 85% and 85% resistant to CIP, LFO and OFL respectively. Isolates of *E. coli* (n=20) collected from the drain downstream to the city were 75%, 70% and 55% were resistant to CIP, LFO and OFL respectively, while the isolates of *S. typhi* (n=20) were 95%, 65% and 70% resistant to CIP, LFO and OFL respectively. This study revealed the occurrence of antibiotics and antibiotic resistant bacteria in hospital wastewater and receiving water bodies, indicating a potential health threat especially in developing countries with no wastewater treatment facility.

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Introduction

Pharmaceuticals has recently been detected from aquatic environment i.e. ground water, surface water, municipal water and also in drinking water. Antibiotics are most important among pharmaceuticals because using for treatment of different type of bacterial infections (Adachi *et al.* 2013). During the last many years, production of pharmaceuticals has rapidly increased.

Approximately 3000 compounds are in use as medicine and annual production reached up to hundreds of tons (Sim *et al.* 2011). It was estimated that worldwide annual consumption of antibiotics is about 100,000 to 200,000 tons (Zheng *et al.* 2011). In 2011 worldwide sale of pharmaceuticals was $ 839 billion (Astra Zeneca 2011) and was $ 950 billion in 2012 (First research 2013). There are about 350 licensed pharmaceutical manufacturers in Pakistan. Annual pharmaceutical market in Pakistan is over $ 1.25 billion (Medinet 2013). Share of antibiotics in the market was approximately $ 140 million during the year 2007 (Shoaib 2007) now it is much more. Antibiotics like ß-lactams, streptomycins and others are produced by soil bacteria in natural environment. But concentration of the antibiotics remains very low or usually below the detection limits (Kummerer 2009), but the release of antibiotics from anthropogenic sources can be up to several mg L^{-1} (Larsson *et al.* 2007).

Common sources responsible for the presence of pharmaceutical compounds in the environment are hospitals, municipal wastewater, livestock farms and pharmaceutical manufacturing units (Zhang *et al.* 2008; Matamoros *et al.* 2008). During the recent years, occurrence, fate and potential toxic effects of antibiotics have become an important research area. Most of the antibiotics are not completely metabolized in human and animals bodies and about 25% to 75% of antibiotics excrete into the environment in active form through urine and feces. So antibiotics used by humans and animals can be found at various concentrations in hospital effluents, municipal wastewater and discharge of wastewater treatment plants. (Brown *et al.* 2006; Zheng *et al.* 2011). As a result receiving water bodies like rivers become contaminated with the antibiotics and the compounds become the part of food chain (Jones-Lepp *et al.* 2012).

The problem of antibiotic resistant bacteria was emerged after the first clinical use of penicillin during the early 1940 (Hiramatsu *et al.* 2012). On the base of research work, Sir Alexander Fleming in an interview (1945) warned that improper use of penicillin could lead to resistant *Staphylococcus aureus* that may cause more severe infections (Alanis 2005). Presence of antibiotics in the environment causes selective pressure on bacterial populations, which lead to resistant bacteria even at low concentrations (Stepanauskas *et al.* 2006). Antibiotic resistant strains belong to pathogenic bacteria can be found from aquatic environment and because of long time exposure to different clinical antibiotics they are resistant to more than one clinical antibiotics (Munir *et al.* 2011). The problem of antibiotic resistant bacteria and their resistant genes is becoming a major global health issue because antibiotic resistant bacteria can distribute resistant genes among microbial community (Schmieder and Edwards 2012). Research is underway to solve the problem by developing new antibiotics. However, soon the bacteria started acquiring resistance to the newly developed drugs and begin to survive as multi drug resistant organism (Hori *et al.* 1993). The situation is more serious in developing countries where water bodies receive untreated wastewater (Ahmad *et al.* 2014) and even in developed countries where rivers are receiving treated effluents contaminated with antibiotics and antibiotic resistant bacteria (Adachi *et al.* 2013). In Pakistan, excess use of antibiotics and antibiotic resistance is sever (Ahmad *et al.* 2017). In developing countries average growth rate of antibiotic resistance is very high, as in China it was 22% in during 1994 to 2000 and it could be the most rapid growth rate of the resistance all over the world (Zhang *et al.* 2006). Wastewater from hospitals are entering into MWW without any treatment and ultimately into receiving water bodies like rivers and lakes etc.

The main aim behind the study was to investigate impact of untreated hospital and municipal wastewater on natural water bodies of Sialkot city. For this purpose wastewater samples from hospital and the drain receiving the combined wastewater were taken to investigate the prevalence of three antibiotics (CIP, LFO and OFL) and bacteria including *E. coli* and *S. typhi* and resistance of these bacteria against the antibiotics under study.

Materials and methods

Analytical grade chemicals and reagents used in this study were purchased from Merck (Germany), Sigma-Aldrich (USA), BDH (UK) and Oxide (UK). Separation columns used in high performance liquid chromatography (HPLC) were also purchased from Merck (Germany).

Solid phase extraction

Before the extraction process the samples were filtered through 0.45µm membrane filter and acidify to pH 3 by adding H_2SO_4. Solid phase extraction (SPE) was carried out by using SPE cartridges (C18) to purify and concentrate the samples. The samples were passed though the cartridges.

To extract the compound of interest 2ml of methanol was passed through the cartridges and collected in a tube. Evaporation of the extracted sample was carried out at 40ºC. At the last step of the extraction process the 1.0ml of 5% aqueous acetonitrile was added to reconstitute the sample. (Lindberg *et al.*, 2004; Brown *et al.* 2006; Shao *et al.* 2009).

Sample analysis

Analysis of the extracted sample was carried out by using high performance liquid chromatography (UFLC Shimadzu, Japan) equipped with diode array detector (SPD M20A).

The analytical column RP-18e (250mm×4.6mm, 5µm) was used at room temperature for analysis. Acetonitrile and H_2O with 1% (v/v) formic acid were used as mobile phase at flow rate of 0.8 ml min^{-1} (Lindberg *et al.* 2004; Seifrtova *et al.* 2009).

Bacterial analysis

For bacteria identification water samples were diluted up to 10^2 to 10^4 times by serial dilution method. From prepared samples 0.1ml was transferred to two types of petriplates containing Brilliance *Salmonella* agar and Brilliance *E. coli*/coli form selective agar for identification and isolation of, *S. typh* and *E. coli* respectively. To spread the sample properly on selective growth media, the plates were rotated clockwise and anti-clockwise and the plates were incubated at 37ºC for 24h. In the petriplates containing Brilliance *E. coli*/coliform selective agar, number of purple colored *E. coli* colonies appeared and *S. typhi* on Brilliance *Salmonella* agar were also appeared as purple colored colonies. (Clark *et al.* 1958; Luczkiewicz *et al.* 2010; Koczura *et al.* 2012).

Different concentrations of CIP, LFO and OFL were prepared by using Iso-sensitest broth. One ml (10^3 CFU/ml) of each bacterium inoculum was transfer to each concentration. The cultures were incubated at 37ºC for 24h. Optical density (OD) of the culture was measured by adjusting the wavelength at 600nm after 0 and 24h. Values of minimum inhibitory concentration (MIC_{90}) for the three bacteria according to specific antibiotics were used to study the tolerance level of the pathogens. The isolates which were able to increase cell population in antibiotic concentrations equal to or higher than the MIC_{90} were considered to be resistant (Roychoudhury *et al.* 2001; Joosten *et al.* 2008; Bidlas *et al.* 2008; Huang *et al.* 2012). Each experiment was run in duplicate.

Results

During the study temporal variation in the concentration of antibiotics was observed in the wastewater of the hospital. Minimum concentration of antibiotics was observed during 15:00 to 19:00 hours and maximum concentration was detected during 11:00 to 15:00 hours (Table 1). Temporal variation in concentration of pharmaceutical compounds in wastewaters is common phenomenon, and it is due to different factors, including retention time in body, used amount of a medicine, and excretion rate (Roig 2010; Gerrity *et al.* 2011). Concentration of the three antibiotics in the hospital wastewater was ranged from 63µg L^{-1} to 189µg L^{-1} and concentration of CIP, LFO and OFL was not same. This variation in concentration is due to different factors including number of patients, liking and disliking of physicians, availability and usage of a drug etc. (Schuster *et al.* 2008; Ort *et al.* 2010). No antibiotic under study was detected from the seasonal drain (locally known as Aik) upstream to first MWW drain but all the three antibiotics were detected downstream to the first MWW drain entering from the city, and concentration was ranged from 0.073 to 0.091µg L^{-1}. The concentration of the pollutants was increased up to 0.18µg L^{-1} downstream to the city. This is because main source of pharmaceuticals are MWW drains (Zhou *et al.* 2011) so antibiotics not usually detected from rivers upstream to point sources (Brown *et al.* 2006; Jones-Lepp *et al.* 2012) and cities (Yang and Carlson 2003).

In second portion of the study two bacteria *E. coli* and *S. typhi* were isolated from hospital effluents and from the drain water understudy. It is worthwhile that *E. coli* and *S. typhi* were not detected from the water samples collected from upstream to the city while both were present in the samples collected downstream to first MWW drain from the city. Hospital effluent isolates (n=20) of *E. coli* (60% to 90%) and *S. tyhi* (85% to 95%) were resistant to concentration equal to MIC_{90} of CIP, LFO and OFL (Fig. 1 to 6). Raw hospital wastewater isolates are usually more resistant to antibiotics (Duong *et al.* 2008) because of patients with severe infections. (Prado *et al.* 2008; Rosenblatt-Farrell 2009). The drain isolates (n=20) of *E. coli* (55% to75%) and *S. typhi* (70% to 95%) were resistant to CIP, LFO and OFL were able to survive at concentration higher than MIC_{90} (Fig. 6 to 12). Main source of the resistant bacteria for water bodies are treated and untreated wastewater (Huang *et al.* 2012) and the problem of antibiotic resistant pathogenic bacteria is more severe in less developed countries (Isturiz and Carbon 2000). From the results it is evident that situation is quite serious, so ongoing studies in local areas should be conducted to investigate further sources and faith of antibiotics and antibiotic resistant bacteria in the environment.

Table 1. Concentration of antibiotics in hospital wastewater and seasonal drain receiving from Sialkot city.

	Antibiotic in hospital wastewater			Antibiotics in MWW/seasonal drain	
	11:00 to 15:00	15:00 to 19:00	19:00 to 23:00	Downstream to first MWW drain	Downstream to the city
CIP	189±0.26	74±0.65	157±0.76	0.091±0.002	0.18±0.031
LFO	142±0.38	89±1.32	119±0.94	0.078±0.0015	0.13±0.034
OFL	116±0.45	63±0.85	93±0.31	0.073±0.0025	0.15±0.023

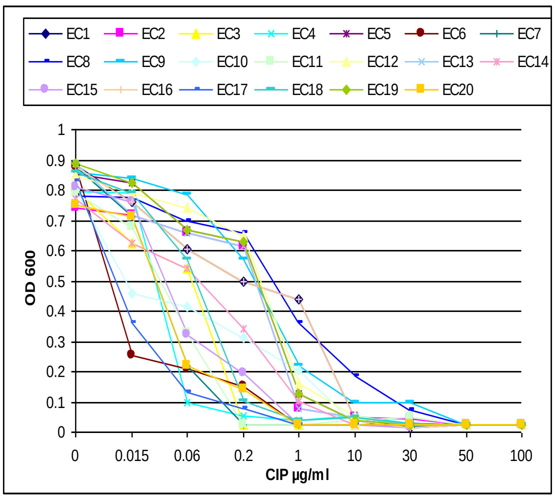

Fig. 1. Resistance of *E. coli* isolates against CIP isolated from hospital effluent.

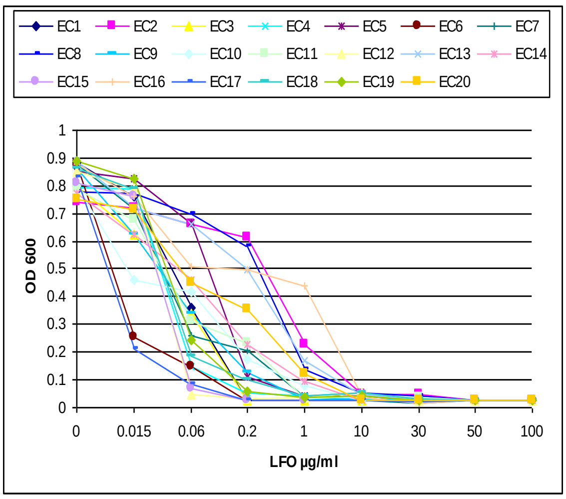

Fig. 2. Resistance of *E. coli* isolates against LFO isolated from hospital effluent.

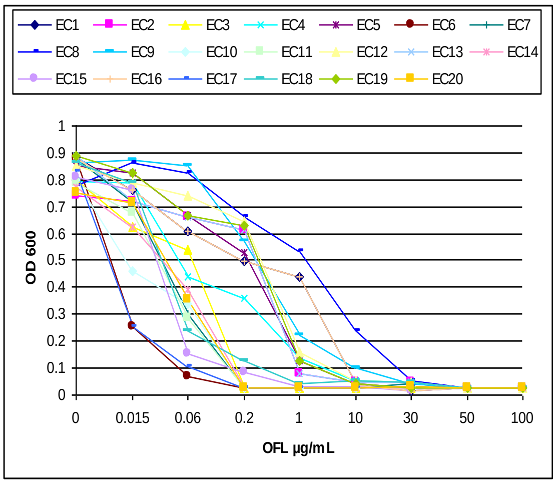

Fig. 3. Resistance of *E. coli* isolates against OFL isolated from hospital effluent.

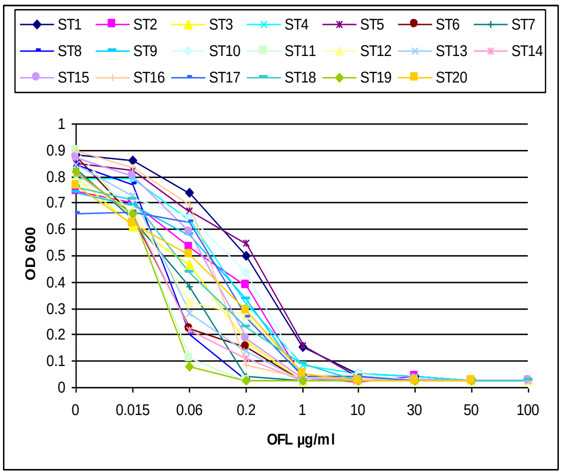

Fig. 6. Resistance of *S. typhi* isolates against OFL isolated from hospital effluent.

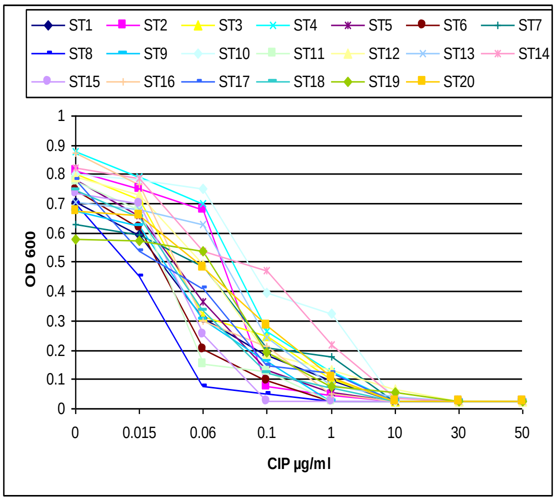

Fig. 4. Resistance of *S. typhi* isolates against CIP isolated from hospital effluent.

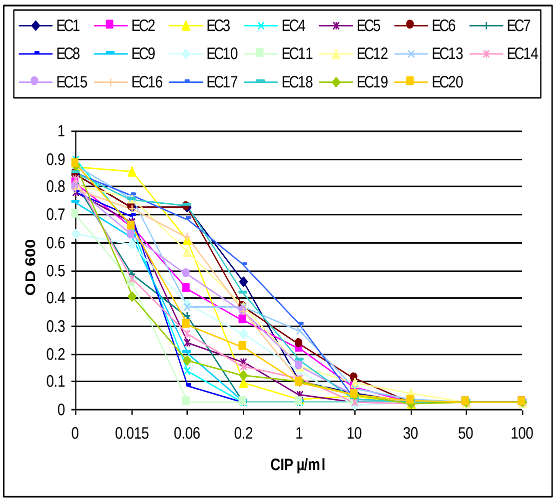

Fig. 7. Resistance of *E. coli* isolates against CIP isolated from the drain.

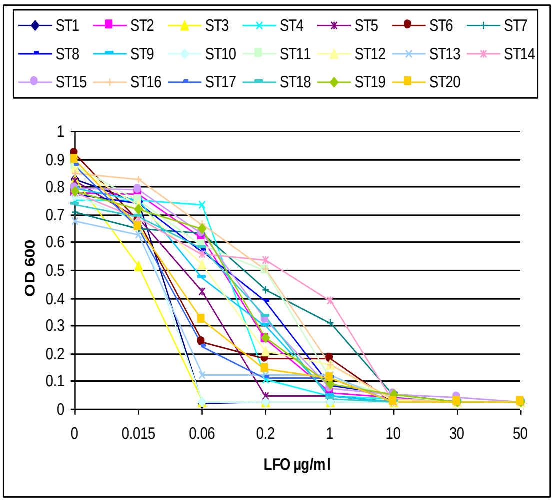

Fig. 5. Resistance of *S. typhi* isolates against LFO isolated from hospital effluent.

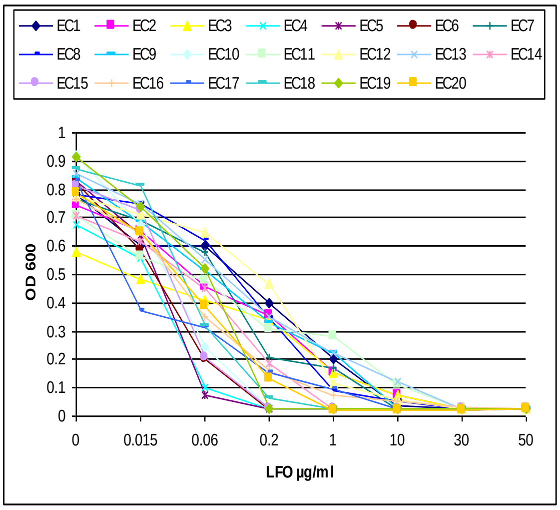

Fig. 8. Resistance of *E. coli* isolates against LFO isolated from the drain.

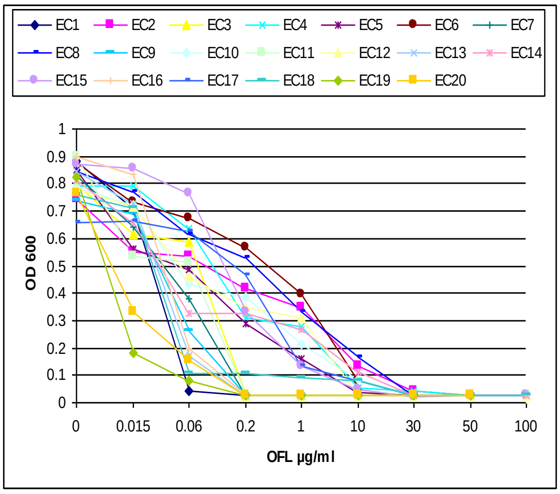

Fig. 9. Resistance of *E. coli* isolates against OFL isolated from the drain.

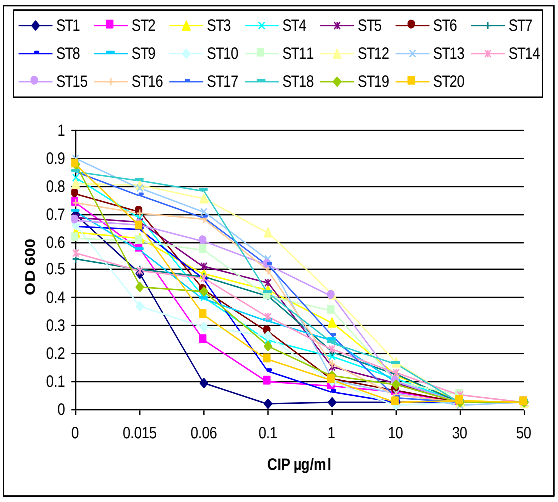

Fig. 10. Resistance of *S. typi* isolates against CIP isolated from the drain.

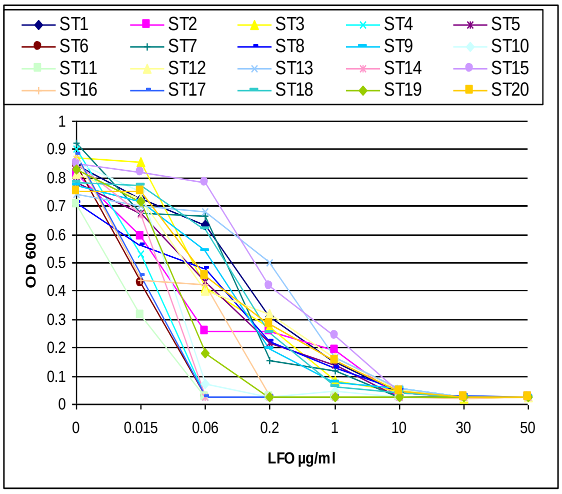

Fig. 11. Resistance of *S. typhi* isolates against LFO isolated from the drain.

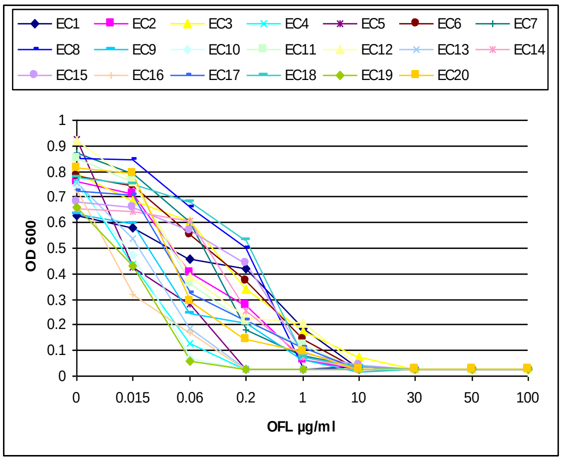

Fig. 12. Resistance of *S. typhi* isolates against OFL isolated from the drain.

References

Adachi F, Yamamoto A, Takakura KI. 2013. Kawahara, R. Occurrence of fluoroquinolones and fluoroquinolone-resistance genes in the aquatic environment. Science of the Total Environment **444,** 508-514.

Ahmad M, Kamran M, Farhan M, Butt ZA, Raza N, Ashraf M, Wahid A. 2017. Prevalence of Multi Drug Resistant Bacteria in Rawal Lake and Its Catchment Area-An alarming situation for Rawalpindi city. International Journal of Biosciences **10(6),** 216-222.

Ahmad M, Khan AU, Wahid A, Butt ZA, Farhan M, Nawaz M. 2014. Assessment of Antibiotics and Antibiotic Resistant Bacteria in Hospital and Municipal Wastewaters. Journal of Pure And Applied Microbiology **8(3),** 2039-2045.

Alanis AJ. 2005. Resistance to Antibiotics: Are We in the Post-Antibiotic Era? Archives of Medical Research **36,** 697-705.

Bidlas E, Du T, Lambert RJW. 2008. An explanation for the effect of inoculum size on MIC and the growth/no growth interface. International Journal of Food Microbiology**, 126,** 140-152.

Brown KD, Kulis J, Thomson B, Chapman TH, Mawhinney DB. 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Science of the Total Environment **366,** 772-783.

Clark HE, Geldrich EF, Kabler PW, Huff CB. 1958. Applied microbiology. International book company, New York 27-33.

Duong HA, Pham NH, Nguyen HT, Hoang TT, Pham HV, Pham VC, Berg M, Giger W, Alder AC. 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. Chemosphere **72,** 968-973.

Gerrity D, Trenholm RA, Snyder SA. 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Research **45,** 5399-5411.

Hiramatsu K, Igarashi M, Morimoto Y, Baba T, Umekitab M, Akamatsu Y. 2012. Curing bacteria of antibiotic resistance: reverse antibiotics, a novel class of antibiotics in nature. International Journal of Antimicrobial Agents **39,** 478-485.

Hori S, Ohshita Y, Utsui Y, Hiramatsu K. 1993. Sequential acquisition of norfloxacin and of loxacin resistance by methicillin-resistant and -susceptible *Staphylococcus aureus*. Antimicrobial agents chemotherapy **37,** 2278-2284.

Huang JJ, Hu HY, Lu SQ, Li Y, Tang F, Lu Y, Wei B. 2012. Monitoring and evaluation of antibiotic-resistant bacteria at a municipal wastewater treatment plant in China. Environment International **42,** 31-36.

Isturiz RE, Carbon C. 2000. Antibiotic use in developing countries. Infection Control and Hospital Epidemiology **21,** 394-397.

Jones-Lepp TL, Sanchez C, Alvarez DA, Wilson DC, Taniguchi-Fu RL. 2012. Point sources of emerging contaminants along the Colorado River Basin: Source water for the arid Southwestern United States. Science of the Total Environment **430,** 237-245.

Joosten H, Marugg J, Stephan R, Klijn A, Jackson T, Iversen C. 2008. A rapid and reliable alternative to ISO 21528-1:2004 for detection of Enterobacteriaceae. International Journal of Food Microbiology **125,** 344-346.

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

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

Larsson DGJ, Pedro C, Paxeus N. 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. Journal of Hazardous Materials, **148,** 751-755.

Lindberg R, Jarnheimer PA, 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 **57,** 1479-1488.

Luczkiewicz A, Jankowska K, Fudala-Ksiazek S, Olanczuk-Neyman K. 2010. Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Research **44,** 5089-5097.

Matamoros V, García J, Bayona M. 2008. Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. Water Research **42,** 653-660.

Munir M, Wong K, Xagoraraki I. 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Research **45,** 681-693.

Ort C, Lawrence MG, Reungoat J, Eaglesham G, Carter S, Keller J. 2010. Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. Water Research, **44,** 605-615.

Prado T, Pereira WC, Silva DM, Seki LM, Carvalho APDA, Asensi MD. 2008. Detection of extended-spectrum-b-lactamase-producing Klebsiella pneumonia in effluents and sludge os a hospital wastewater treatment plant. Letters in Applied Microbiology **46,** 136-141.

Roig B. 2010. Pharmaceuticals in the environment: Current knowledge and need assessment to reduce presence and impact. IWA Publishing Alliance House, UK pp. 180.

Rosenblatt-Farrell N. 2009. The landscape of antibiotic resistance. Environmental Health Perspective, **117(6),** A244-A250.

Roychoudhury S, Catrenich EC, Mcintosh EJ, Mckeever HD, Makin KM, Koenigs PM, Ledoussal B. 2001. Quinolone resistance in *Staphylococci*: Activities of new nonfluorinated quinolones against molecular targets in whole cells and clinical isolates. Antimicrobial Agents and Chemotherapy **45(4),** 1115-1120.

Schmieder R, Edwards R. 2012. Insights into antibiotic resistance through metagenomic approaches. Future Microbiology **7(1),** 73-89.

Schuster A, Hadrich C, Kummerer K. 2008. Flows of active pharmaceutical ingredients originating from health care practices on a local, regional, and nationwide level in Germany - is hospital effluent treatment an effective approach for risk reduction? Water Air and Soil Pollution **8,** 457-471.

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

Shao B, Chena D, Zhanga J, Wub Y, Sun C. 2009. Determination of 76 pharmaceutical drugs by liquid chromatography-tandem mass spectrometry in slaughterhouse wastewater. Journal of Chromatography **A1216,** 8312-8318.

Shoaib MH. 2007. Pharmacokinetic and physico-chemical studies on oral cephradine preparations. Ph.D Thesis, Department of Pharmaceuticals, University of Karachi.

Sim WJ, Lee J, Lee ES, Shin SK, Hwang SR, Oh JE. 2011. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. Chemosphere **82,** 179-186.

Stepanauskas R, Glenn TC, Jagoe CH, Tuckfield RC, Lindell AH, King CJ. 2006. Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. Environmental Microbiology **8,** 1510-1514.

Yang S, Carlson K. 2003. Evolution of antibiotic occurrence in a river through pristine urban and agricultural landscapes. Water Research **37,** 4645-4656.

Zhang R, Eggleston K, Rotimi V, Zeckhauser RJ. 2006. Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States. Globalization and Health **2,** 6.

Zhang YJ, Geiben SU, Gal C. 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere **73,** 1151-1161.

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

Zhou LJ, Ying GG, Zhao JL, Yang JF, Wang L, Yang B, Liu S. 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. Environmental Pollution **159,** 1877-1885.