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OCCURRENCE, FATE AND ECOLOGICAL RISK OF ANTIBIOTICS IN HOSPITAL EFFLUENT WATER AND SEDIMENTS IN SRI LANKA

Gayani Yasodara Liyanage[1] and Pathmalal M. Manage[1*]

Centre for Water Quality and Algae research, Department of Zoology,
University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250,Sri Lanka

ABSTRACT

Antibiotics are among the emerging micro contaminants in the aquatic environment due to their potential adverse effects on the ecosystem and possibly on human health. Four important antibiotic classes, sulfanomides [sulfadiazine (SDI), sulfamethoxazol (SMX)], penicillin [amoxicillin (AMX), ampicilline (AMP)], tetracycline [oxytetracycline (OTC), tetracycline (TET)] and macrolids [erythromycin (ERM)] are used for human and veterinary medicine, were studied. Triplicate samples of hospital effluents water and sediment were collected from 50 sampling sites in different area of the country and Horton plaines as pristine environment for the study. Solid-Phase Extraction (SPE) was employed to concentrate anibiotics and quantification of were done by High Performance Liquid Chromatography (HPLC). Recoveries for each antibiotic was remained between 83% ± 0.021 to 95% ± 0.034. Among the selected antibiotics tested; the highest concentrations of AMX and AMP were recorded in hospital effluent water. AMX and AMP were detected range between 0.001-0.023 ppm and 0.001 – 0.024 ppm respectively. The other antibiotics concentrations were; TET (water: 0-0.001ppm, sediments: N.D); SDI (water: 0.001- 0.003ppm, sediments: .001-0.003ppm); SMX (water: 0.001- 0.018ppm, sediments: 0.001-0.002ppm); ERM (water: 0.001-0.008ppm, sediments: 0.001-0.003ppm) respectively. Relatively high removal efficiency was detected for TET (50-100%) where desending order was followed by SDI (63-72%), SMX (52-72%), ERM (48-100%), AMP (40-54%) and AMX (35-58%) respectively. The results of the study can be incorporated into environmental risk assessments of the particular contaminants as the published information regarding antibiotic contamination ststus in water and sediment are limited in Sri Lanka.

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Keywords: Amphicillin (AMP); Amoxicilline (AMX); Sulfadiazine (SDI); Sulfamethoxazol (SMX); Oxytetracycline (OTC); Ttetracycline (TET); High Performance Liquid Chromatography (HPLC).

1.0 INTRODUCTION

Antibiotics are used extensively in human and veterinary therapy, aquaculture, for the purpose of preventing or treating microbial infection. The term "antibiotic" originally referred to any agent with biological activity against living organisms; however, "antibiotic" now refers to substance with antibacterial, antifungal or antiparasitical activity" (Hirsch et al. 1999). There are about 250 different chemical entities registered as antibiotics for use in human and veterinary therapy at present (Moges et al. 2014).

The first antibiotic discovered from fungi and now synthetic antibiotics such as sulfa group and semi-synthetic antibiotics like cephalosporin group are used (Kummerer 2009). It was estimated that worldwide annual consumption of antibiotics by human was about 100 000 to 200 000 tons per capita and the individual share of compound varies from country to country (Kummerer 2009). Antibiotic prescription rates and intake without prescription vary markedly between countries (Molstad et al. 2002). Data on the country-specific groups of antibiotics are varying in different countries but mostly as Defined Daily Dose (DDD) according to WHO (WHO 2013). However, at present, there are no discharge standards for antibiotics were adopted in Sri Lanka. Proper treatment facilities; even in hospitals were not properly established. Therefore, excessive use of antibiotics may lead to occurrence of traces of chemical compounds in the aquatic environment (Schmidt et al. 2000; Ferrari 2003).

Antibiotics can been more or less extensively metabolized by human, animals and finally reach to the environment (Ritter et al. 2008). After administration to human body, some antibiotics excreted as it is or as other form of metabolites into the environment in different ways (Ritter et al. 2008). Important point is the non-metabolized fractions of antibiotic is excreted as a still active compound (Ritter et al. 2008) and only partially eliminated during the treatment processes, through the sewage system and may end up in the aquatic and soil compartment of the environment. Residual amounts can contaminate sediments, surface and ground water as well (Giorgia et al. 2004).

The antibiotics; sulfanomides, penicillin, tetracycline and macrolids were most often prescribed antibiotics for common infectious diseases in both human and animals. Sulfadiazine (SDI) and Sulfamethoxazol (SMX) are sulfanomides which are a group of antibacterial agents commonly used in urinary tract infections, pneumocystis pneumonia, chronic branches and etc (BNF 2010), where Amoxicilline (AMX) and Ampicilline (AMP) are penicillins which have been most

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widely used antimicrobial drugs for more than 80 years and still considered as one of the most important group of antibiotic. Cloxacillin is used to treat different types of infectious diseases caused by Staphylococcus bacteria ("staph" infections). Oxytetracycline (OTC) and tetracycline (TC) are characterized by a broad spectrum activity against pathogenic microorganisms (Thomas et al. 2007). Erythromycin (ERM) is commonly used extensively as a major alternative to penicillins and cephalosporins in many countries for the treatment of infections, especially those caused by β-haemolytic Streptococci and Pneumococci (Catherine et al. 2007).

The heavy use of antibiotics in farm animals, plant protection, veterinary and human medicine in unregulated manner may increase the antibiotic contaminations in the surrounding environment. During the recent years antibiotics have categorized as high risk pollutants in the environment because of their toxic effects on bacteria and algae at very low concentrations and also due to their toxic capacity to create resistant among natural bacteria populations (Wellington et al. 2013). Ataee et al. (2012) detected amoxicillin (AMX) in different types of wastewater in Lahore and documented high concentration of AMX in hospital effluent (7.31–39.13 ppb), municipal wastewater (0.54–1.29 ppb) and in River Ravi (0.14–0.37 ppb) respectively . High concentration of ciprofloxacin (218–236 g/L) sulfamethoxazole (4.6 g/L), oxytetracyclin (3.2 g/L) and trimethoprim (2.2g/L) were reported in hospital wastewater in India (Hughes et al. 2013).

The antibiotic detection in aqueous samples is largely related to persistence and transport in the aquatic environment. In fact, sulfanomide, flouroquinolones and macrolids antibiotics showed the highest persistence and were frequently detected in hospital effluent (Huang et al. 2001). The likelihood of detection depends on both the compound's initial effluent concentration and its environmental persistence (Huang et al. 2001).

Thus, intensive use of antibiotics has caused general concern about the risk for development of bacteria that are resistant to such chemical substances (Kummer. 2009). In most of aquatic environments the concentrations of antibiotics have recorded as very low concentrations (Hughes et al. 2013). However, residual antibiotics in the environment have tendency to convert nonpathogenic bacteria to pathogenic bacteria by increasing the resistance against antibiotics (Kim et al. 2012).Taubes (2008) and Satoru et al. (2008) documented that the continuous exposure even to low concentrations of antibiotics can produce resistant strains of bacteria in the environment .

Antibiotic resistance determinants can also be transmitted to other animals and humans via consumption of polluted water and it may lead to global public health problem due to development antimicrobial-resistant bacteria. According to the recent WHO report approximately 290,000 new Multi Drug-Resistant Tuberculosis (MDR-TB) cases have been

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recorded globally and one third of which may prove fatal (WHO 201; Aminov and Mackie 2007). An advanced level of MDR-TB, extensively drug resistant TB (XDR-TB), has also been identified in 77 countries worldwide (Aminov and Mackie 2007; Babu and Ramana 2012).

Moreover, resistant bacterial infectionse specially in hospitals have been caused more than 25,000 mortality in a year in Europe (WHO 2013). These public health risks, based on microbial drug resistance, which have affected the cost and productivity of healthcare (WHO 2013; Rizzo et al. 2013).

As the authors ar aware, limited information regarding antibiotic contamination status are available in Sri Lanka. In the present study, it was observed that most of hospital discharging points do not comply with environmental regulations and discharge wastewater directly to the natural environment or into domestic sewage network without any treatment.

Thus, the present study recorded contamination status of some selected antibiotics (AMX,AMP, OTC, TET, SDI, SMX, ERM) present in water and sediments of hospital wastewater discharges as base line data. Further, it was studied differences between antibiotic concentrations in influent and effluent at hospital effluent water treatment plants (WWTP) in some selected hospitals. Thus, the results will provide reference for the risk control of antibiotic resistance bacteria, antibiotic resistance genes in natural environment and help authorities to take action for improve proper treatment facilities for effluent water. According to the authors' knowledge; this is the first report of the presence of residual contamination status of antibiotics in Sri Lanka.

2.0 MATERIALS AND METHODOLOGY

2.1 Standards and Reagents

Two tetracyclines including Oxytetracycline (OTC) (97%), Tetracyclines (TET) (97%) ,two sulfanomides including Sulfadiazine (SDI) (96%), Sulfamethoxazon (SMX) (97%), three penicillins including Amoxicillin (AMX) (96%), Ampicilline (AMP) (97%), Erythromycin (ERM) (96%) and all the HPLC grade chemicals were purchased from Sigma Aldrich, USA.

2.2 Sample collection and physico-chemical analysis

Hospital effluent water and sediment samples were collected from 50 sampling locations in different districts in Sri Lanka (Figure 1). Among those locations, influent and effluent water at Waste Water Treatment Plant (WWTP) were collected from 17 hospitals. Samples from Horton plains and Bakers' fall were collected and considered as pristine environment for the study. Triplicate water and sediment samples were collected from each location following satandard procedure and GPS points of the sampling locations were recorded (GPS Model: Etrex 30,

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Taiwan). Sediment samples were collected from 0-3cm depth at each location and transferred into UV sterilized black poly propylene bags. All the samples were transported to the laboratory in ice box and kept at 4^0C until analysis (Satoru et al. 2008).

Water temperature, pH and DO of samples were measured at the site itself using a thermometer (Model: Philip Haris, England), pH meter (Model: pH3110, WT Co., Weilheim, Germany), and DO meter (Model: Cond3110, WT Co., Weilheim,. 53 Germany) respectively (APHA,2013).

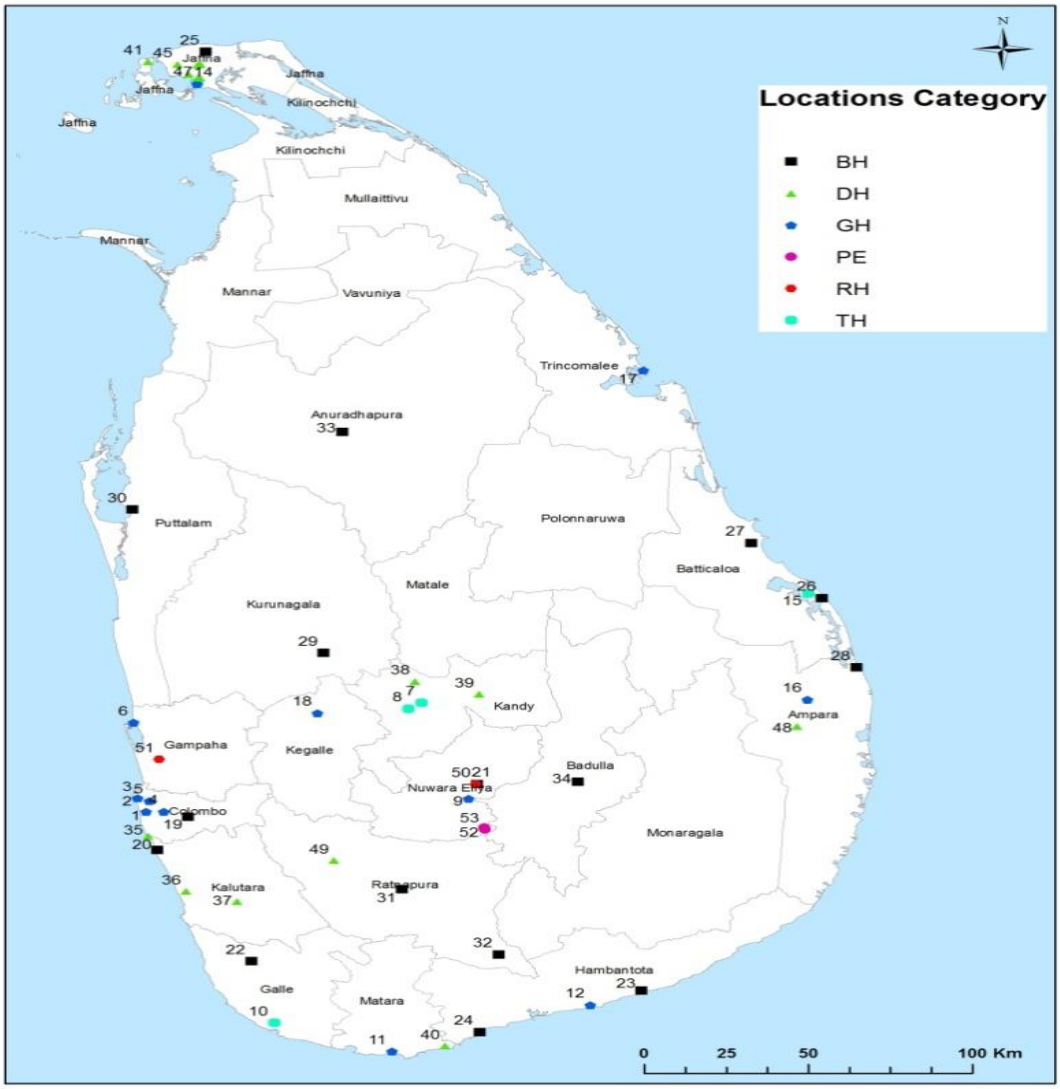

Figure 1: Sampling locations at the study

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Sampling location	No	Sampling location	No
CSTH	1	BH Valaichchenai	27
General-Colombo	2	BH-Kalmunai	28
Nawaloka	3	BH-Kurunagala	29
J'pura hospital	4	BH-Puttalam	30
Kasal Hospital	5	BH-Kahawatta	31
Negombo-GH	6	BH-Ambilipitiya	32
Teaching H.Kandy	7	BH-Anuradhapura	33
TH-Peradeniya	8	BH-Badulla	34
GH-Nuwaraeliya	9	Moratuwa DH	35
Karapitya	10	Kaluthra-DH	36
GH-Matara	11	Mathugama-DH	37
Hambanthota-GH	12	Akurana-DH	38
TH-Jaffna	13	DH(Madadumbara)	39
GH-Jaffna	14	DH-Dickwella	40
TH Batticaloa	15	DH Karainagar	41
GH-Ampara	16	DH Konadavil	42
GH-Trincomallee	17	DH Kokuvil	43
GH-Kegalle	18	DH Manipay	44
Homagam BH	19	DH Vaddukkoddai	45
Panadura-BH	20	DH Chunnakam	46
Ambewala Hospital	21	DH Uduvil	47
BH-Alpitiya	22	DH-Damana	48
Ambalanthota-BH	23	DH-Rathnapura	49

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Tangalle-BH	24	RH-Lindula	50
BH Tellippalai	25	Jae la-RH	51
BH Kattankudy	26	Horton plaines	52

2.3 Recovery test

The recovery tests were carried out for each antibiotic; 1 L of sterilized distilled water sample was spiked with the antibiotic at final concentration of 3000ppm. Sterilized 100g of sediment sample was dissolved in 1 L of sterilized deionized water and spiked with respective antibiotic at final concentration of 3000ppm and sonicated at 35 KHz, centrifuged for 10 min at 5300 rpm in order to collect supernatant. The samples were subjected to Solid Phase Extraction (SPE) by using C18 cartridge (Baquero et al., 2008). pH for the recovery test was optimized as pH 3 for the maximum recovery (> 85) of the selected antibiotics.

2.4 Antibiotic analysis

1L wastewater sample was adjusted to pH 3 and the sample was filtered through 0.22µm nuclearpore filter. Sediment sample was dissolved in 1L of sterilized distilled water, sonicated at 35KHz and centrifuged for 10 min at 5300 rpm and the resultant supernatant was collected. Water and supernatant of sediment samples were spiked with antibiotic at final concentration of 100ppm and subjected to SPE (Baquero et al. 2008).

The C18 cartridges were preconditioned with 5ml 100% methanol and then 5ml double-deionized water. Pre-prepared samples were then passed through the C18 cartridges at a flow rate of approximately 1-2ml/min and then rinsed with 5ml of deionized water. The analytes were eluted with 5ml of 100% methanol and then 2ml of 100% isopropanol. The extracts were purged by nitrogen stream and redissolved with 1ml of mobile phase (Liyanage and Manage 2014; Pérez-Burgos 2012).

The target antibiotics were quantified by using Agilent 1200 series High Performance Liquid chromatography (HPLC) equipped with diode array and fluorescence detector. Antibiotics in water and sediment samples were analyzed according to the Fernandez et al. (2010) with a modification. 0.1% glacial acetic acid (polar protic solvent) was used as mobile phase in the present study. The injected volume was 20µl and chromatography was performed at 30^0C. The mobile phase of the mixture of 0.1% Glacial acetic acid in water (Component A): 0.1% Glacial

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acetic acid in acetonitrile (Component B), 99:1 (v/v) was pumped beginning at a flow rate of 0.7 ml/min for OTC, TC, AMP, SDI, SMX, AMX and ERM. Then followed linear elution gradient from 99% to 70% A in 25 min for antibiotics (Liyanage G.Y and Manage P.M 2014; Pérez-Burgos 2012). Table 2 shows conditions and retention times employed for each antibiotic analysis.

Table 1: Monitoring wave lengths and retention times for each antibiotic tested in the present study

Analyte	λ absorption (nm)	Retention time (min)	SD (min) (n=3)
OTC	280	17.34	0.002
TET	280	16.56	0.001
AMP	230	13.08	0.001
AMX	230	11.23	0.006
SDI	300	12.56	0.003
SMX	280	18.12	0.002
ERM	250	14.56	0.005

2.5 Calibration plots

Individual calibration standards of each antibiotic (OTC,TCs, AMP, SDI, SMX, AMX, ERM) at 0- 5000 ppm were prepared. The linearity of the method was evaluated by plotting peak area as function of the concentration of each analyte.

2.6 Determination of antibiotic removal percentage

The antibiotic concentration of influent and effluent water of hospital treatment plants were quantified by using the method described in 2.4. The antibiotic degradation percentages were calculated using the equation given below, where "C_o" and "C" are the concentration of antibiotics at the influent and at the effluent respectively.

$$\text{Antibiotic removal percentage (\%)} = \frac{C_0 - C}{C_0} \times 100$$

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3.0 RESULTS

3.1 Physico-chemical parameters

Temperature of the effluent water was ranged between 18.3±0.002^{0}C - 32.7±0.002^{0}C. The lowest temperature was record in the Hortain plaines (18.3±0.16^{0}C) and the highest was in Jaffna (32.7±0.002^{0}C) respectively. DO values were ranged between 3.4-5.5mg/l and pH remained in 5.6-8.2 except effluent water collected from the hospital in Kahawatta (9.03±0.11) and Kurunagala (8.82±0.09). The results showed that the physio-chemical parameters of the sampling sites were within the ranges given for aquatic life (WHO 2013).

3.2 Calibration curves

The concentration of OTC, TCs, AMP, SDI, SMX, AMX and ERM were analyzed using the equations given in table 2. Equations were derived from the calibration plots (Table 2) for each antibiotic and the peak area of the sample, (eg: - peak area of OTC = A_{OTC}), and the slope and intercept of each calibration curve was used to calculate the concentration of antibiotic in unknown samples (eg:- concentration of OTC =C_{OTC}).

Table 2: Derived equations for antibiotics quantification

Name of Antibiotic	Derived Equation
Oxytetracycline (OTC)	$C_{OTC} = (A_{OTC} – 9.917)/ 0.158$
Tetracycline (TC)	$C_{TC} = (A_{TC} – 7.345) / 0.149$
Ampicillin (AMP)	$C_{AMP} = (A_{AMP} – 15.9)/ 0.394$
Amoxicillin (AMX)	$C_{AMX} = (A_{AMX} – 16.3)/ 0.257$
Sulfadiazine (SDI)	$C_{SDI} = (A_{SDI} – 1.234)/ 0.32$
Sulfamethaxazol (SMX)	$C_{SMX} = (A_{SMX} – 12.4)/ 0.145$

Table 3: Recovery percentages for antibiotics; OTC, TC, SDI, SMX, AMX, AMP and ERM

Sample type	Recovery (%)						
	OTC	TCs	SDI	SMX	AMX	AMP	ERM
Water	90± 0.002	87± 0.001	92± 0.011	83± 0.001	88± 0.008	85± 0.012	92± 0.025
Sediments	88± 0.005	84± 0.004	85± 0.032	86± 0.004	91± 0.027	83± 0.002	87± 0.001

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A rapid, simple and specific method for the quantification of the OTC, TCs, AMP, SDI, SMX, AMX, and ERM in environment samples was modified according to Fernandz et al. (2010). As given in the table 3, more than 83% recoveries were obtained for antibiotics (OTC,TCs, AMP, SDI, SMX, AMX, and ERM) in both water and sediments.

The lowest recovery for water was detected for the SMX (83± 0.001) and other were remained greater than 85 ± 0.012. The recovery of antibiotic was ranged between 83- 92% for water and 83-91% for sediments respectively

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Table 4. Antibiotic contamination levels in hospital effluent water and sediment (n=3)

Sampling Sites	Antibiotic concentration (ug/ml)													
	OTC		TET		AMP		AMX		SMX		SDI		ERM	
	water	Sed	water	Sed	water	Sed	water	Sed	water	Sed	water	Sed	water	Sed
Teaching Hospitals (TH)														
Colombo Southern Teaching Hospital (CSTH)	N.D	N.D	N.D	N.D	**0.004±0.097**	**0.001±0.023**	**0.009±0.002**	N.D	N.D	N.D	N.D	N.D	**0.002±0.012**	N.D
Colombo	N.D	N.D	N.D	N.D	**0.001±0.005**	N.D	**0.003±0.021**	**0.001±0.007**	N.D	N.D	N.D	N.D	**0.001±0.009**	**0.001±0.017**
Nawaloka	N.D	N.D	N.D	N.D	**0.001±0.032**	N.D	**0.001±0.024**	N.D	**0.001±0.031**	N.D	N.D	N.D	N.D	N.D
J'pura hospital	N.D	N.D	N.D	N.D	**0.006±0.011**	**0.003±0.007**	**0.002±0.011**	**0.005±0.021**	N.D	N.D	N.D	N.D	N.D	N.D
Kasal Hospital	N.D	N.D	N.D	N.D	**0.001±0.088**	N.D	**0.001±0.042**	N.D	N.D	N.D	**0.001±0.102**	N.D	N.D	N.D
.Kandy	N.D	N.D	N.D	N.D	**0.005±0.078**	0**.004±0.011**	**0.012±0.001**	**0.007±0.032**	N.D	N.D	**0.001±0.011**	N.D	**0.004±0.014**	N.D
Peradeniya	N.D	N.D	**0.001±0.021**	N.D	**0.003±0.043**	**0.001±0.005**	**0.009±0.034**	**0.002±0.011**	N.D	N.D	N.D	N.D	N.D	N.D
Jaffna	N.D	N.D	N.D	N.D	**0.023±0.004**	N.D	**0.022±0.009**	**0.005±0.012**	**0.034±0.007**	0.001± 0.021	N.D	N.D	**0.005±0.002**	**0.001±0.003**
Batticaloa	N.D	N.D	N.D	N.D	**0.013±0.014**	**0.001±0.015**	**0.015±0.007**	**0.003±0.011**	N.D	N.D	N.D	N.D	**0.008±0.022**	N.D
Karapitya	N.D	N.D	N.D	N.D	**0.001±0.022**	N.D	**0.001±0.046**	N.D	N.D	N.D	N.D	N.D	N.D	N.D
General Hospitals (GH)														
Negombo	N.D	N.D	N.D	N.D	**0.001±0.023**	N.D	N.D	**0.002±0.013**	N.D	N.D	N.D	N.D	**0.001±0.134**	N.D
Nuwaraeliya	N.D	N.D	N.D	N.D	N.D	N.D	**0.002±0.062**	N.D	N.D	N.D	**0.001±0.034**	N.D	N.D	N.D

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Matara	N.D	N.D	**0.001±0.003**	N.D	**0.002±0.006**	N.D	**0.004±0.008**	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Hambanthota	N.D	N.D	N.D	N.D	**0.014±0.001**	**0.001±0.003**	**0.023±0.021**	**0.004±0.032**	**0.001±0.029**	N.D	**0.001±0.043**	**0.001±0.054**	**0.004±0.034**	**0.001±0.038**
Jaffna	N.D	N.D	N.D	N.D	**0.017±0.009**	**0.014±0.014**	**0.024±0.021**	**0.001±0.034**	**0.004±0.015**	N.D	**0.003±0.023**	**0.001±0.016**	**0.006±0.018**	**0.002±0.011**
Ampara	N.D	N.D	N.D	N.D	**0.002±0.011**	**0.001±0.008**	**0.001±0.015**	N.D	**0.001±0.012**	N.D	N.D	N.D	**0.002±0.004**	N.D
Trincomallee	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kegalle	N.D	N.D	N.D	N.D	N.D	N.D	**0.021±0.005**	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Base Hospitals														
Homagam	N.D	N.D	N.D	N.D	**0.002±0.043**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	**0.001±0.055**	N.D
Panadura	N.D	N.D	N.D	N.D	N.D	N.D	**0.003±0.001**	N.D	**0.001±0.046**	N.D	N.D	N.D	N.D	N.D
Ambewala	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Alpitiya	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Ambalanthota	N.D	N.D	N.D	N.D	**0.002±0.017**	**0.003±0.005**	**0.005±0.002**	**0.001±0.018**	**0.001±0.018**	N.D	**0.001±0.024**	**0.001±0.005**	**0.002±0.011**	**0.002±0.008**
Tangalle	N.D	N.D	N.D	N.D	**0.018±0.005**	**0.009±0.101**	**0.019±0.084**	**0.001±0.012**	**0.001±0.097**	N.D	N.D	N.D	N.D	N.D
Tellippalai	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kattankudy	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Valaichchenai	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	**0.001±0.001**	N.D	N.D	N.D
Kalmunai	N.D	N.D	N.D	N.D	N.D	**0.001±0.009**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kurunagala	N.D	N.D	N.D	N.D	N.D	N.D	**0.004±0.006**	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Puttalam	N.D	N.D	**0.001±0.014**	N.D	**0.004±0.018**	**0.001±0.012**	**0.007±0.005**	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kahawatta	N.D	N.D	N.D	N.D	**0.003±0.007**	N.D	**0.004±0.008**	N.D	N.D	N.D	N.D	N.D	N.D	**0.001±0.013**

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Ambilipitiya	N.D	N.D	N.D	N.D	N.D	N.D	N.D	**0.001±0.023**	N.D	**0.002±0.021**	N.D	N.D	N.D	N.D
Anuradhapura	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Badulla	N.D	N.D	N.D	N.D	**0.003±0.087**	**0.001±0.023**	N.D	**0.001±0.027**	N.D	**0.001±0.043**	N.D	N.D	**0.002±0.028**	**0.001±0.017**
Divisional Hospitals														
Moratuwa	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kaluthra	N.D	N.D	N.D	N.D	**0.005±0.003**	**0.001±0.001**	**0.008±0.001**	**0.002±0.001**	**0.001±0.001**	**0.001±0.003**	N.D	N.D	N.D	N.D
Mathugama	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Akurana	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Madadumbara	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Dickwella	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Karainagar	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Konadavil	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Kokuvil	N.D	N.D	N.D	N.D	N.D	N.D	**0.001±0.045**	**0.003±0.019**	N.D	N.D	N.D	N.D	N.D	N.D
Manipay	N.D	N.D	N.D	N.D	**0.001±0.006**	N.D	N.D	N.D	**0.001±0.006**	N.D	N.D	N.D	**0.001±0.004**	N.D
Vaddukkoddai	N.D	N.D	N.D	N.D	**0.011±0.004**	**0.002±0.002**	**0.013±0.003**	**0.001±0.005**	N.D	N.D	N.D	**0.003±0.003**	N.D	N.D
Chunnakam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Uduvil	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Damana	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Rathnapura	N.D	N.D	N.D	N.D	**0.002±0.006**	**0.001±0.008**	**0.002±0.004**	N.D	N.D	N.D	N.D	N.D	**0.001±0.015**	N.D
Regional Hospitals														
Lindula	N.D	N.D	**0.001±0.008**	N.D	**0.003±0.021**	**0.002±0.042**	**0.001±0.057**	**0.001±0.028**	N.D	N.D	N.D	N.D	**0.005±0.004**	**0.003±0.002**
Ja ela	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pristine environment														
Hortain plaines-1	N.D		N.D		N.D		N.D		N.D		N.D		N.D	N.D

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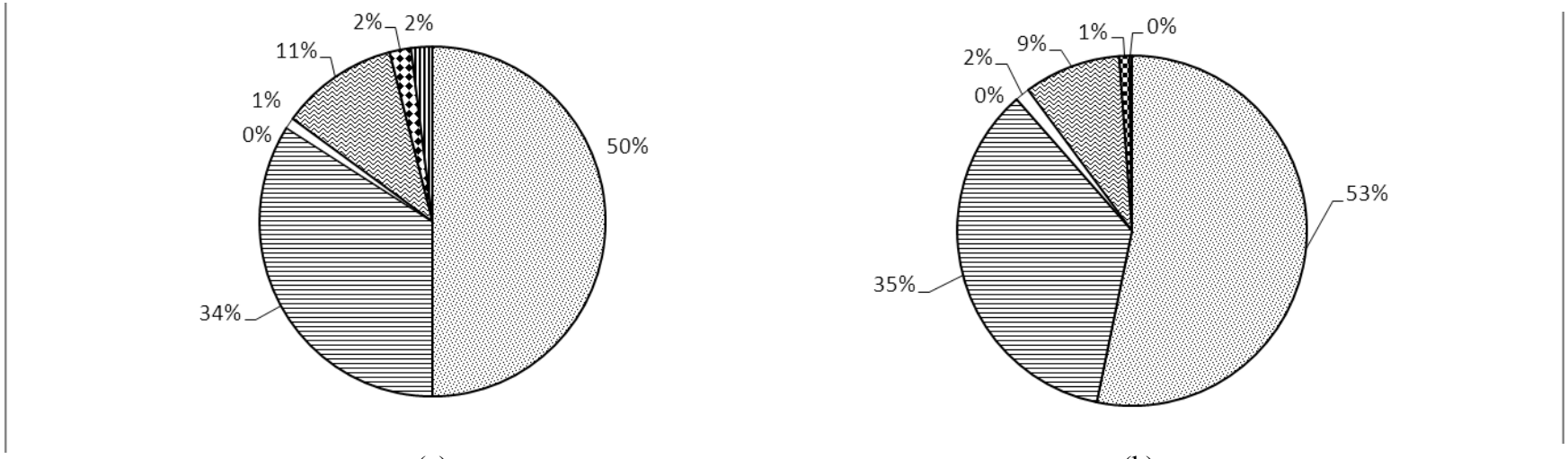
Bakers fall-1	N.D		N.D		N.D		N.D		N.D		N.D		N.D	N.D

Values expressed as mean ± SD

N.D; Not detected / below the detectable level



(a) (b)

Figure 2: Total antibiotics percentages in hospital effluents and sediments; (a) Effluent water , (b) Sediments AMX AMP OTC TET SMX SDI ERM

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Concentrations of antibiotics in hospital effluent water and sediment are given in table 4. Six out of 7 antibiotics were detected in samples of effluent water and sediments (Table 4). Specially, AMX and AMP were detected more than 30 samples where ERM in 23 samples, SMX in 18 samples, SDI in 7 samples and TET in 5 samples respectively. Overall, 40 out of 52 samples were contaminated at least with one antibiotic (Table 4).

Among the antibiotic groups, penicillin (AMX, AMP) was recorded at high concentration in hospital effluent where as AMX and AMP were detetcted 0.001 to 0.024 ppm and from 0.001 to 0.023 ppm range (Table 4) respectively. In contrast, low concentrations of antibiotics belonging to the group tetracycline, OTC (N.D) and TC (~ 0.001ppm) were detected (Table 4).

Among the selected antibiotics, approximately 50% from the AMX and 34% from the AMP contributes to the environmental pollution through the hospital effluents were estimated while other antibiotics (SDI- 2.07%, SMX- 10.62 %, ERM – 2.07%, TET- 0.92%) contributes at low percentages (Figure 2). Similarly, AMX (53.24%) and AMP (35.28%) in sediments were recorded as high contributers on environmental pollution whereas TET (1.35%), OTC (N.D), SMX (8.95%) , SDI (0.94%) and ERM (0.24%) contribution is comparatively low percentages (Figure 2). Such high contaminations of AMX and AMP in hospitals wastewater may due to improper discard of antibiotics, as the penicillin group is frequently used to treat many infectious diseases (Pérez-Burgos et al. 2007; Taubes 2008).

The maximum concentrations of the AMX (water: 0.024ppm, sediments: 0.005 ppm), AMP (water: 0.023ppm, sediments: 0.014 ppm) and SDI (water: 0.003ppm, sediments: 0.003 ppm) were detected in samples collected from Jaffna district hospitals (Figure 1). The considerable concentration of ERM in water (0.008 ppm) and sediments (0.003ppm) were detected in effluent water at Batticalo and Ambilipitiya hospitals as well (Table 4).

Thus, results of the present study showed that environmental contamination by TET (water: ~ 0.001ppm, sediments: N.D); SDI (water: 0.001- 0.003ppm, sediments: .001-0.003 ppm); SMX (water: 0.001- 0.018ppm, sediments: 0.001-0.002ppm); ERM (water: 0.001-0.008ppm, sediments: 0.001-0.003ppm) may lead to antibiotic resistance in the environment (Table 4). Interestingly, non of antibiotic was detected in samples collected from Hortoin plaines (Table 4), where situated in the highest latitude of present sampling locations.

The WHO has recommended less than 0.001 ppm of antibiotic residues in the aquatic environment and less than 0.1 ppm in soil respectively (Connor and Aga 2007; NRDC 2014). The results of the study showed that the detected antibiotics levels in effluent water were exceeded the recommended values given by the WHO (water; <0.001ppm, sediments; <0.1ppm) except OTC. Furthermore, the high concentrations raises question concerning effective levels

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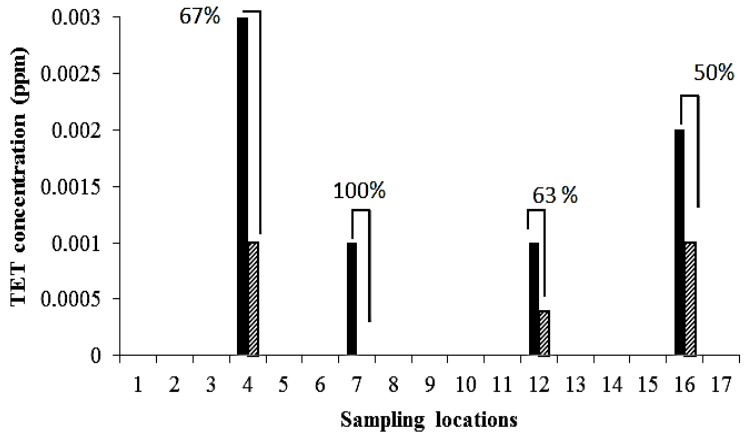
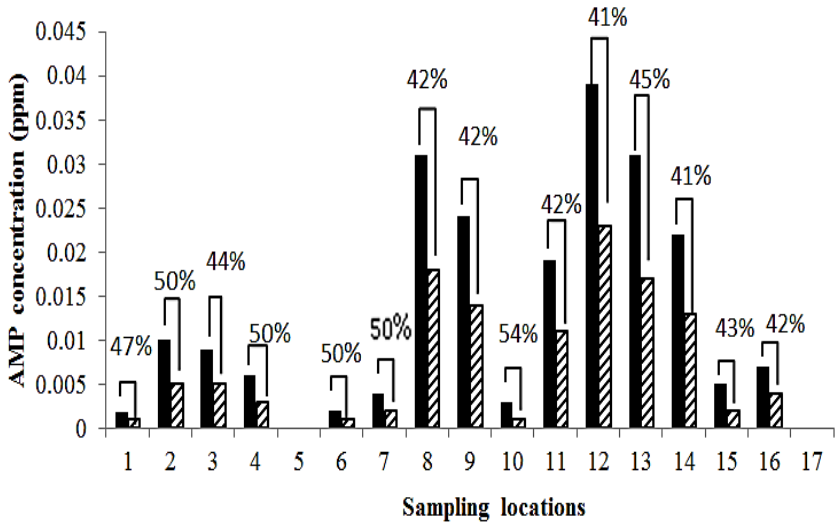
and risk assessments. It is well known that it may possible to develop antibiotic-resistant bacteria in the environment (Connor and Aga 2007; Li et al. 2012).

However, the concentration of antibiotics detected in sediments was below the maximum permissible level given by the WHO (< 0.1ppm) (Table 4).



(a)



(b)

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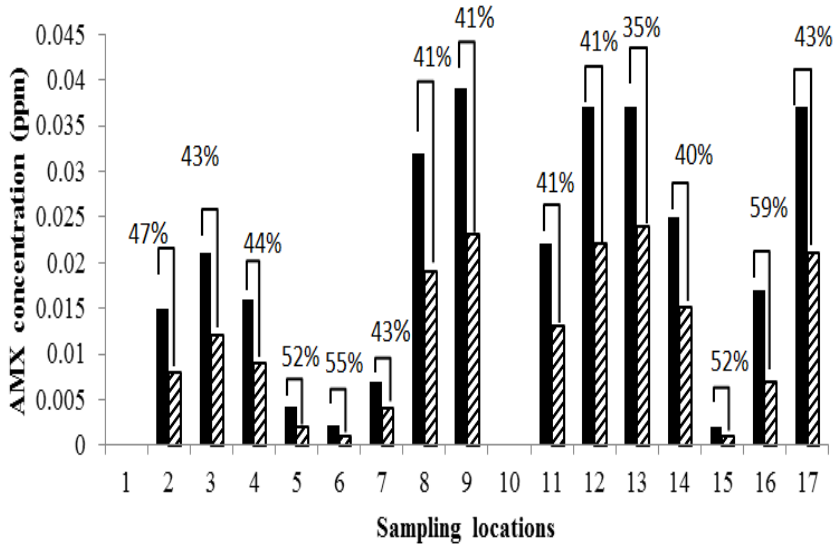
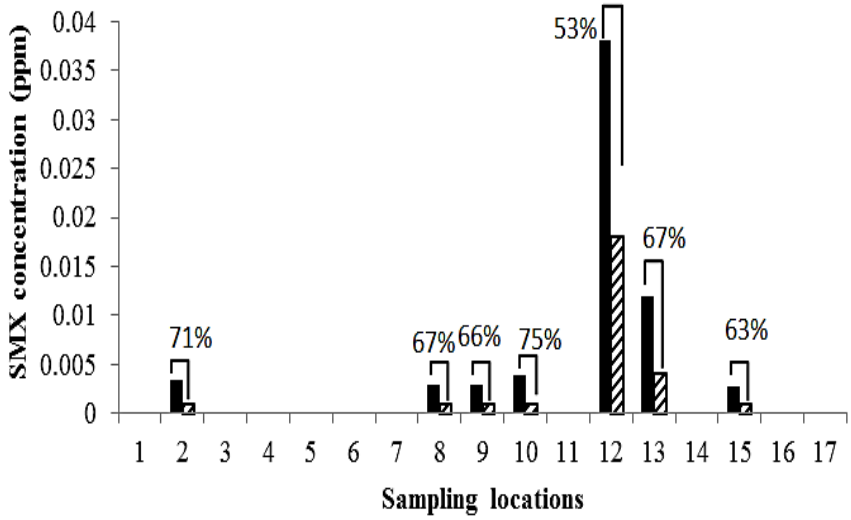


(c)



(d)

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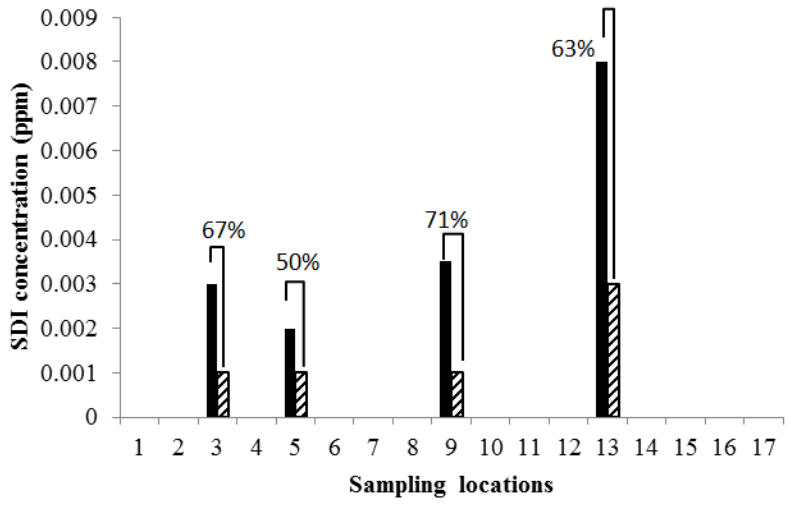
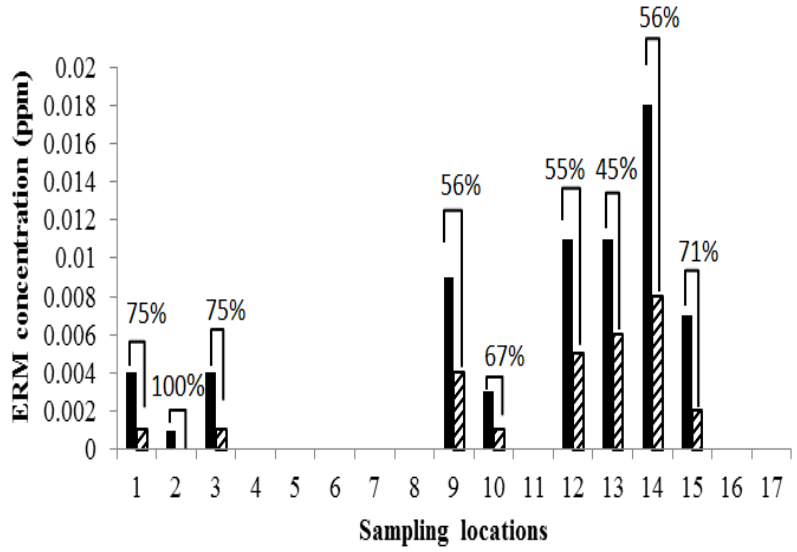


(e)



(f)

Figure 3: Comparison of antibiotic concentrations in influent and effluent wastewater in treatment plants in hospitals and antibiotic removal percentages.(a) TET (b) AMP (c) AMX (d) SMX (e) SDI (f) ERM [1-Negombo (GH), 2-Kandy (TH), 3- Peradeniya (TH), 4-Nuwara eliya (GH), 5- Karapitiya (TH), 6- Matara (GH), 7- Hambanthota (GH), 8- Jaffna (TH), 9- Jaffna (GH), 10- Kegalle (GH), 11- Batticalo (TH), 12- Ampara (GH), 13-Kaluthara (DH), 14- Manipay (DH), 15- Vaddukodai (DH), 16- Tangalle (BH), 17-Puttalam (BH)]

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(Closed bar:- Influent in WWTP, hatched bar:- effluent from WWTP)

The removal percentages of selected antibiotics in the wastewater treatment plants in 17 hospitals are given in figure 3. It was calculated that contaminations of TET, SDI, SMX and ERM were decreased to more than 60% in the hospital effluents before released to the environment (Figure 3). Overall, decrease percentages were determined for the whole treatment proces were ranged between 50-100% ,40-54%, 35-58%, 52-72%, 63-72% for TET, AMP, AMX, SMX, SDI and ERM respectively. The removal efficiency of different classes of antibiotic was different and followed the order tetracyclines > sulfanomides > macrolids > penicillines.

It was detetcted that antibiotics such as AMX, AMP, ERM, SMX in hospital effluent remain considerabal high concentration even after treatment process where TET and OTC remain less concentration after the treatment. Non of antibiotic was completely removed after the treatment was recorded.

DISCUSSION

During the last many years, production of pharmaceuticals increased rapidly and approximately 3000 compounds are in use as medicine and annual production reached up to hundreds of tons (Suga et al. 2013). Among the large number of pharmaceutical compounds antibiotics are important because of their wide use in humans, animals, multiple purposes such as growth promoter and etc. This may lead to enhance the ability to produce resistant bacteria in the environment (Pradhan and Mishra 2010).

In the present study parent compounds of the 7 antibiotics were studied and results revealed that relatively high concentrations of antibiotics are present in the environment and their primary contributers are hospitals. Huang et al. (2001), documented that the drug classes of penicillin and sulfanomides were well represented in hospital effluents by the presence of AMX, AMP, SMX in many effluent samples and this was true for the present study as well (Table 4) . The results of the present study comparable to the findings of Brown el al. (2006), who reported concentrations of SDI (0.0004 -0.0021 ppm), AMX (0.0008 -0.0052ppm) , OTC (not detected), SMX (not detected) in hospital effluents in New Mexico . The maximum concentrations of penicillin (0.001 – 0.029ppm) and SMX (0.001-0.028ppm) reported in the study was correlated with results published by Ahmed et al. (2012), who recorded high concentrations of ofloxacin (0.005 - 0.015ppm), ciprofloxacin (0.004 – 0.009 ppm), sulfamethoxazol (0.011 – 0.023 ppm) and penicillin (0.007- 0.035ppm) in hospital effluents in Pakistan.

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Kolpin et al. (2002) monitored the antibiotics' residues of TCs (0.003-0.023ppm) and sulfanomides (0.004-0.011ppm) in 139 hospitals in the United States and warned these dangerous concentration levels as ecological aspect. They explained that the wastewater of hospital effluent may be a major source of antibiotics' release into water systems in United States. In another study' chlorotetracyclines, AMP, ERM and SUF were detected at levels ranging from 2 to 540 µg/L in effluent water from Swine hospitals, while TET and OTC were not detected (Rodriguez- Mozaz et al. 2015).

Similarly to previous studies conducted in different part of the world, effluent of hospitals were a significant source of antibiotics (OTC, TET, AMP, AMX, SUF, SMX, ERM) detected at the present study and the concentrations were ranging from 0.001- 0.034ppm. AMX and AMP were the most frequently detected antibiotics among 7 antibiotics tested in hospital effluent samples. It was found that 40 samples out of 50 was positive for AMX and AMP in Sri Lanka (Table 4).

The results of the present study showed low antibiotic contamination levels in sediment (Table 4). Similarly, Kim et al. (2012) also recorded low concentrations of TCs (0.001ppb-0.003ppb) [including chlortetracycline (CTC) and oxytetracycline (OTC)] and SAs (0.002-0.005 ppb) [including SMX and sulfathiazole (STZ)] in sediments collected from the Cachela Poudre River in northern Colorado, the United States. Li et al. (2012) studied the contamination status of antibiotics in penicillin group (AMX, AMP) and recorded concentrations were lower in soil than water . This may due to sorption behavior of soil, soil pH, soil organic matter, soil particle size distribution, metal oxide content, photolysis ability, ionic strength and microbial remediation activity (Martinez 2008; McManus et al. 2002). Thus, the reported low concentrations of antibiotics in sediment samples may due to the factors mentioned (Martinez 2008; McManus et al. 2002).

Pristine reference sample was collected from Horton plains which situated 2100 m above sea level and decleared as World Heritage Sites (WHS) by UNESCO (UNESCO 2010). The investigators of the present study assumed that the Horton plain is free of antibiotic contamination due to minimum anthropological impact and strick rules and regulation practices for human entry and carrying food, drinks and medicins with visitors to the Horton plain. Interestingly, non of antibiotics were detected in Horton plaines and the assumption is justifiable as pristine environment for the study.

Previous studies have recorded that SMX (Martinez 2008; McManus et al. 2002), Oxacillin (OXA), chloramphenicol, quinolones trimethoprim are heat-stable (Singer et al., 2003) while OTC and ERM are heat-labile (Hsieh et al. 2011). On the other hand, several ß –lactams such as AMP and AMX appear partially heat-liable (Slinn. 2009). Invitro studies revealed that higher percentage reduction of AMX and AMP were apparent at higher temperature. Upto 99% at

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121ºC, 54.4% at 100ºC for most antibiotics including AMX, AMP and ERM were recorded (Slinn. 2009). In previous realvent studies have shown that the high temperature (> 35^0C) and alkalinity pH (> 8.2) affected on reduction of antibiotics in natural environment (Awad et al. 2015; Hsieh et al. 2011). In the present study physiochemical parameters such as water temperature (23-32^0C), pH (5.8 – 8.2) and DO (3.6 – 5.5 ppm) were not changed in a wide range and the recorded ranges were may not affected to the reduction of antibiotics in the environment (Table 4).

It is important to realize that it is not essential for an antibiotic to be persistence in the environment in order to be detected. Instead, an antibiotic could be present at concentrations of concern when continual infusion into the environment (Cabello 2006). Consequently, the potential long-term effect of relatively low concnetrations of antibiotics in environment needs to be paid much more attention as this may enhance antibiotic resistant within the microbial community (Silbergeld et al. 2008).

In the present study the removal percentages of tetracyclines in hospital water treatment plants (~65% to 100%) were greater than the other antibiotics and comparable to the levels reported in other studies elsewhere (Li et al. 2010; Zhang and Li 2011). Zhou. et al. (2013) reported similar elimination percentage (75-95%) for tetracyclines and suggested the removal may due to limited applications of human therapy, biodegradation and adsorption (Yasser et al. 2014; Zhou et al. 2013). Further Smith et al. (2002) documented that tetracyclines are light sensitive and sorb significantly to soils or sewage sludge (kim et al. 2012). The present study, it was detected that sulfanomides removal percentages was between 65% to 75% (Figure 2). This may due to sulfanomides are not easy to degrade and hydrophilic enough to transfer into the aquatic environment (Sharma et al. 2016). Sharma et al. (2016) reported low reduction of sulfanomides (40-55%) compared to the tetracyclines (75-90%) as well. The present study sulfanomides removal efficiency was ranged between 60% to 75% (figure 2) and removal percentages of penicillines were low (~ 40 -55%) compared with the other antibiotics. In Italy, the mean degrees of AMP and AMX elimination were estimated to be 75% and 80 % (De la torre et al. 2012). The difference of removal efficiency was related to the differences in the chemical properties and structure of antibiotic (Napolitano et al. 2013). Therefore. in general, WWTPs could not completely remove antibiotics and this may resulting discharge of antibiotics into the environment even after treatment (Koyalova et al. 2013). Advance wastewater treatment techniques such as reverse osmosis, activated carbon and ozonation have been shown to significantly reduce or eliminate antibiotics including sulfanomides from effluent water (Zhang and Li. 2011); however, such wastewater treatment facilities are not accessible all part of the world due to high cost.

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The widespread and often inappropriate administration of antibiotics in livestock and hospitals have shown to result in the development of antibiotic resistant bacteria and is generally accepted to be a primary pathway for their proliferation in the environment (Angulo et al. 2004). There is further concern that antibiotic-resistant bacteria might develop from long-term environmental exposure to low concentrations of antibiotic (ng/L -µg/L), such as those present in wastewater and surface water (Awad et al. 2015). As a result of the continuous exposure to antibiotic residues in the environment, multidrug resistant pathogens can make drugs ineffective and pose a serious risk to the global pharmaceutical and healthcare industry (Hijosa-valsero et al. 2011).

Thus, detailed and targeted studies are required to investigate the sources, pathways and fate of antibiotics and their microbial resistance in the entire environment. Therefore the baseline data collected in the present study will provide first-hand information to establish more advance wastewater treatment facility inorder to reduce antibiotic contamination which will safe guard human and other animals health.

CONCLUSION

The present study established a preliminary inventory of antibiotic occurrence in effluent from hospitals in Sri Lanka. Among the selected antibiotics; the highest concentrations of AMX (0.001-0.023ppm) and AMP (0.001 – 0.024ppm) were recorded in hospital effluent. The other antibiotics contamination were followed; TET (water: 0-0.001ppm, sediments: N.D); SDI (water: 0.001- 0.003ppm, sediments: .001-0.003ppm); SMX (water: 0.001- 0.018ppm, sediments: 0.001-0.002ppm); ERM (water: 0.001-0.008ppm, sediments: 0.001-0.003ppm) respectively. As expected, hospitals were a significant source of antibiotics and the values were exceeded than the guideline given by WHO. Therefore further studies are required to study the ways to enhance the removal efficiency of antibiotics in the aquatic environment.

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REFERENCES

1. Ahmad, Maqsood., Amin U. Khan, Abdul Wahid, Zahid Ali Butt, Muhammad Farhan & Farooq Ahmad. (2012). Role of hospital effluents in the contribution of antibiotics and antibiotic resistant bacteria to the aquatic environment. Journal of Nutrition, 12, 1177-1188 .

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2. Ataee, R.A., Mehrabi-Tavana A., Hosseini S.M.J., Moridi K.Ghorbananli. & Zadegan M. A. (2012). A method for antibiotic susceptibility testing: Applicable and accurate. Jundishapur Journal of Microbiology, 5(1), 341–345.
3. Aminov, R. I. & R. I. Mackie. (2007). Evolution and ecology of antibiotic resistance genes. FEMS Microbiol Lett. 271(2), 147-161.
4. Angulo, F.J., Nargund, V.N. & Chiller, T.C. (2004). Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J. Vet. Med. B Infect. Dis. Vet. Public Health. 51**,** 374–379.
5. APHA. (2013). https://www.standardmethods.org/ViewArticle.cfm?article ID=98, Accessed 25 November 2014.
6. Awad, Y. M., Kim, K. R., Kim, S. C., Kim, K., Lee, S. R., Lee, S. S., & Ok, Y. S. (2015). Monitoring Antibiotic Residues and Corresponding Antibiotic Resistance Genes in an Agroecosystem. Journal of Chemistry, 501.
7. Babu Giridhara R. & Ramanan Laxminarayan. (2012). The unsurprising story of MDR-TB resistance in India. Tuberculosis. 92 (4), 301-306.
8. Baquero F., Martinez J.L., & Canton. R. (2008). Antibiotics and antibiotic resistance in water Environments. Current Opinion Biotechnology. 18, 123-134.
9. British National Formulary. (2010). http://www.amazon.co.uk/British-National-Formulary-March-2010/dp/0853699291 . Accessed 18 June 2015.
10. Brown Kathryn D., Jerzy Kulis, Bruce Thomson, Timothy H. Chapman. & Douglas B. Mawhinney. (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 (2), 772-783.
11. Cabello, F.C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ.Microbiol. 8**,** 1137–1144.
12. Catherine V. (2007). Hawkyard & Roland J. Koerner The use of erythromycin as a gastrointestinal prokinetic agent in adult critical care: benefits versus risks, J. Antimicrob. Chemother. 59 (3), 347-358.
13. Connor S.O. & Aga D.S. (2007). Analysis of tetracycline antibiotics in soil; advances in Extraction, clean-up, and quantification. TrAC Trends in Analytical chemistry. 26,456-465.
14. De la Torre, Ana, Irene Iglesias, Matilde Carballo, Pablo Ramírez, & María Jesús Muñoz. (2012). An approach for mapping the vulnerability of European Union soils to antibiotic contamination."Science of the Total Environment . 414 , 672-679.

ISSN: 2455-6939

Volume:02, Issue:04

15. Ferrari B. (2003). Ecotoxicologial impact of pharmaceuticals found in treated wastewaters: Study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicology and Environmental Safety. 55, 359-370.
16. Fernandez-Torres, M.O. Consentino, M.A. Bello Lopez, & J.L. Larsen. (2010). Simultaneous determination of 11 antibiotics and their main metabolites from four Different groups by reversed-phase high performance liquid chromatography-diode array -fluorescence (HPLC-DAD-FLD) in human urine sample, Talanta. 81, 871-880.
17. Giorgia, M.L., Davide, C., Paolo, G., Sara, C., Giuseppe, C. & Roberto, F. (2004). Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. Chemosphere. 54, 661-668.
18. Hirsch, R., Ternes T.,Harberer K. & Kratz K.L. (1999). Occurrence of antibiotics in the aquatic environment, Science Total Environment. 225, 109-118.
19. Hsieh, M. K., Shyu, C. L., Liao, J. W., Franje, C. A., Huang, Y. J., Chang, S. K.. & Chou, C. C. (2011). Correlation analysis of heat stability of veterinary antibiotics by structural degradation, changes in antimicrobial activity and genotoxicity. Vet Med (Praha), 56, 274-285.
20. Hijosa-Valsero, M., Fink, G., Schlüsener, M. P., Sidrach-Cardona, R., Martín-Villacorta, J., Ternes, T., & Bécares, E. (2011). Removal of antibiotics from urban wastewater by constructed wetland optimization. Chemosphere, 83(5), 713-719.
21. Huang, J., Lih, C. J., Pan, K. H., & Cohen, S. N. (2001). Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthetic pathways in Streptomyces coelicolor using DNA microarrays.Genes & Development, 15(23), 3183-3192.
22. Hughes, S.R., Kay, P. & Brown, L.E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47(2), 661-677.
23. Kim, S. J., Ogo, M., Oh, M. J. & Suzuki, S. (2012). Occurrence of tetracycline resistant bacteria and tet (M) gene in seawater from Korean coast. Interdisciplinary studies on environmental chemistry—Environmental pollution and ecotoxicology. TERRAPUB, Tokyo, 367-375.
24. Kovalova, L., Knappe, D. R., Lehnberg, K., Kazner, C. &Hollender, J. (2013). Removal of highly polar micropollutants from wastewater by powdered activated carbon. Environmental Science and Pollution Research, 20(6), 3607-3615.
25. Kolpin, D. W., Furlond, E.T., Meyer, M.T., Zauss, S.D., Barber, L.B. & Buxton, H.T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental science & technology, 36(6), 1202-1211.

ISSN: 2455-6939

Volume:02, Issue:04

26. Kummerer, K. (2009). Antibiotics in the aquatic environment-A review-part I, Chemosphere. 75. 435-441.
27. Li, W., Shi, Y., Gao, L., Liu, J., & Cai, Y. (2012). Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. Chemosphere. 89(11), 1307-1315.
28. Liyanage. G. Y. and Manage. P.M. (2014). Quantification of Oxytetracycline and Amphicillin in two waste water discharging points in Colombo, Sri Lanka, Journal of Environemnt and Natural Resources, Thailand. 12, 129-130.
29. Martinez, J.L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science*. 321, 365–367.
30. McManus, P.S., Stockwell, V.O., Sundin, G.W. & Jones, A.L. (2002). Antibiotic use in plant agriculture. Annu. Rev. Phytopathol.40, 443–465.
31. Moges F., Endris M., Belyhun Y. & Worku W. (2014). Isolation and characterization of multiple drug resistance bacterial pathogens from waste waterin hospital and non-hospital environments,Northwest Ethiopia. Chemosphere. 7, 215-220.
32. Molstad, S., lungborg, C.S., Karlsson, A.K. & Cars, O. (2002). Antibiotic prescription rates vary markedly between 13 European countries. Scand. Infection Diseases. 34, 366-371.
33. Napolitano, F., Izzo, M. T., Di Giuseppe, G., & Angelillo, I. F. (2013). Public knowledge, attitudes, and experience regarding the use of antibiotics in Italy. PloS one, 8(12), e84177.
34. NRDC. (2015). Dosed without prescription; preventing Pharmaceutical contamination of our nation's drinking water. http://www.nrdc.org/health/files/dosed4pgr.pdf. Accessed 22 June 2015.
35. Pérez-Burgos, R., Grzelak, E. M., Gokce, G., Saurina, J., Barbosa, J., & Barrón, D. (2012). Quechers methodologies as an alternative to solid phase extraction (SPE) for the determination and characterization of residues of cephalosporins in beef muscle using LC–MS/MS. Journal of Chromatography *B*, 899, 57-65.
36. Pradhan, B., & Mishra, S. K. (2010). Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. Kathmandu University Medical Journal. 8(1), 40-44.
37. Ritter J.M., Lewis L.D., Mant T.G.K. & Ferro A. (2008). Clinical pharmacology and Therapeutics. Pharmacology. 5, 223-245.
38. Rodriguez-Mozaz, Chamorro, S., Marti, E., Hueta, B., Gros, M., Sanchez-Melsio, A., Barcelo, D. and Balcazar, J.L. (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water research*. 69, 234-242.

39. Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I. & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Science of the total environment. 447, 345-360.
40. Satoru, S., Takeshi, K., Fujiyo, S., Bui CachTuyen & Tough Seang, T. (2008). High Occurrence rate of tetracycline resistant bacteria and TC resistance gene relates to microbial diversity in sediment of Mekong river mail water way. Microbes Environment. 23, 149-152.
41. Schmidt. A.S., Bruun M.S., Dalagaard I, Pedersen K. & Larsen J.L. (2000). Occurrence of Antimicrobial resistance in fish pathogenic and environmental bacteria associated with Four danish rainbow trout farms, Applied and Environmental Microbiology. 66, 4908-4915.
42. Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H. A. (2016). A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere. 150, 702-716.
43. Singer, R.S., Finch, R., Wegener, H.C., Bywater, R., Walters, J., Lipsitch, M. (2003). Antibiotic resistance – the interplay between antibiotic use in animals and human beings. Lancet Infect. Dis. 3, 47–51.
44. Slinn, J. (2009). Robert Bud, Penicillin: triumph and tragedy. Medical History. 53(01**),** 133-134.
45. Silbergeld, E. K., J. Graham & L. B. Price. (2008). Industrial Food Animal Production, Antimicrobial Resistance, and Human Health. Annu. Rev. Public Health. 29, 151–169.
46. Smith, D.L., Harris, A.D., Johnson, J.A., Silbergeld, E.K. & Morris Jr., J.G. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc. Natl. Acad. Sci. 99, 6434–6439.
47. Suga, N., Ogo, M., & Suzuki, S. (2013). Risk assessment of oxytetracycline in water phase to major sediment bacterial community: a water-sediment microcosm study. Environmental toxicology and pharmacology. 36(1), 142-148.
48. Taubes G. (2008). The bacteria fight back. Science. 321(5887), 356-361.
49. Thomas K.V., Dye C, Schlabach. M. & Langfor K.H. (2007). Source to sink tracking of selected human pharmaceuticals from two Oslo city hospitals and a wastewater treatment works, Environmental Monitor, 9, 1410-1418.
50. UNESCO (2010). Central highlands of Sri Lanka., http://whc.unesco.org/en/list/1203. Accessed 21 May 2016.
51. Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M. & Williams, A.P. (2013). The role of the natural environment in the emergence of

ISSN: 2455-6939

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antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases, **13(2),** 155-165.

52. WHO. (2013). Tuberculosis MDR-TB & XDR-TB 2011 Progress Report. http://www.who.int/tb/challenges/mdr/factsheet_mdr_progress_march2011.pdf. Accessed 12 May 2016.
53. Yasser M. Awad, Sung-Chul Kim, Samy A. M. Abd El-Azeem, Kye-Hoon Kim, Kwon-Rae Kim,Kangjoo Kim, ChoongJeon, Sang Soo Lee & Yong Sik Ok. (2014). Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility, Environmental earth sciences. 71(3), 1433-1440.
54. Zhou, L. J., Ying, G. G., Liu, S., Zhao, J. L., Yang, B., Chen, Z. F., & Lai, H. J. (2013). Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. Science of the total environment, 452, 365-376.
55. Zhang, T., & Li, B. (2011). Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. Critical reviews in environmental science and technology, 41(11), 951-998.

