Original Research Article

OCCURRENCE AND REMOVAL EFFICIENCIESOF FOUR ANTIBIOTICS IN KISII AND KABARNET WASTE WATER TREATMENT PLANTS, KENYA

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ABSTRACT

Background: Antibiotics are presently considered as emerging contaminants with adverse effects in the environment and the population such as the development of antimicrobial resistant genes (ARG) and antimicrobial resistant bacteria (ARB). This study was guided by objective entailing to determine the seasonal occurrences and removal efficiencies of four antibiotics in Kisii and Kabarnet waste water treatment plants in Kenya.

Place and Duration of Study: Waste water samples were picked from Suneka wastewater treatment plant in Kisii county and Kabarnet Level V Hospital in Baringo County Kenya in the months of June and December 2020

Methodology: Multiple sampleswere picked in Kisii and Kabarnet waste water treatment plants in the months of June and December correspond to the dry and wet seasons in Kenya. Collected waste water samples were centrifuged and filtered with glass microfiber filter papers and subsequently passed through a Solid phase extractor cartridge. High Performance Liquid Chromatography was used for quantification of antibiotics as per international commission for harmonization and subsequently applied for analysis.

Results: The results revealed that the dry season had higher antibiotic concentrations at 1.29, 0.09, 2.92 and 1.82 µg/l for sulphamethoxazole, trimethoprim, ampicillin and amoxicillin respectively for the Kisii waste water treatment plant and 0.18, 0.05, 1.34 and 0.09 µg/l respectively for Kabarnet waste water treatment plant. During the wet seasons the measured concentrations were 1.11, 0.14, 2.04 and 1.34 µg/l for sulphamethoxazole, trimethoprim, ampicillin and amoxicillin at the Kisii WWTP, and 0.14, 0.06, 1.01 and 0.09 µg/l for Kabarnet WWTP. The removal efficiencies in the WWTPs, ranged from a high of 94 % to a low of 11.11 % depending on the type of antibiotic in both wet and dry seasons.

Conclusion: Amoxicillin, ampicillin, trimethoprim and sulphamethaxazole were found in both Kisii WWTPs and Kabarnet WWTPs with nearly all antibiotics having a higher concentration of in the dry season than in the wet season. The highest calculated percentage removal was 94.03 % while the lowest calculated percentage removal was 7.14 %. The results suggest that the two WWTPs are effective for the removal of different types of antibiotics.

Keywords: Antibiotics, removal efficiency, seasonal variation, HPLC,

1. INTRODUCTION

Considered amongst the class of emerging contaminants, antibiotics, are a group of antimicrobial drugs whose mode of action involves the killing or inhibition of bacterial growth. Currently, antibiotics are widely used in both veterinary medicine and human medicine for the reduction and elimination of infectious diseases with an estimated worldwide consumption of more than 200,000 tons annually [1,2]. In the past decades, the demand and indiscriminate antibiotics consumption has led to an alarming increase of antibiotics in the environment. These residues, persist in the environment including the various stages of waste water treatment plants (WWTPs) posing an environmental threat to the ecosystem, health, and potentially contributing to the rise in antibiotic resistance [1,3].

There are several pathways in which antibiotics can enter fresh and subsequently waste water systems such as direct disposal of expired and unused drugs, hospital effluents, animal husbandry, aquaculture, antibiotic manufacturing plants, underground spillage etc[2, 4, 5]. The main problem being that most WWTPs are not designed for the treatment or removal of pharmaceutical drugs leading a subsequent direct discharge into the environment especially in cases where there is a direct reuse of water [6]. Although most developed nations have re-engineered their WWTPs systems for the elimination and removal of emerging contaminants, most African countries including Kenya, are yet to do so.The major risks of antibiotics residues in waste water treatment plants is due to the potential development of antimicrobial resistant genes (ARG) and antimicrobial resistant bacteria (ARB) in the environment [7]. In fact research has shown that the sludge in WWTPs, can accumulate antibiotics posing a great danger to the environment especially to crop consumers of stabilized sludge amended soils [8,9]. Currently, AMR and ARG pose a major threat to the general public health and to the overall safety of patients due to its ability to resist drugs leading to elevated costs of treatment, needless deaths and lack of proper and effective treatment therapies [10,11].This study therefore avails data concerning the occurrence and the removal efficiencies two major waste water treatment plants in Kisii and Kabarnet county headquarter.

2. MATERIAL AND METHODS

2.1 Chemicals

High-purity standards for trimethoprim (TRI), ampicillin (AMP), sulfamethoxazole (SMX), and amoxicillin (AMX) manufactured by Sigma-Aldrich Germany were sourced and supplied by Kobian Laboratories Kenya. Analytical grade and high-performance liquid chromatography (HPLC)-grade water, methanol, and acetonitrile for extraction and analysis, Solid-phase extraction cartridgesOasis MCX cartridges and nylon micro filters were obtained from Estec Kenya Limited. All the stock solutions were made using HPLC-grade methanol. Chromatographic solvents were filtered through a 0.22μm nylon membrane filter (Fioroni Filters, Ingré, France) using a vacuum pump (Dinko D-95, Barcelona, Spain). The solvents were degassed for 15 min in an ultrasonic bath (Sonorex Digital 10P, Bandelin DK 255P, Germany).

2.2 Sampling

Waste water samples were picked from Suneka wastewater (0° 39' 30” S, 34° 42' 30” E) treatment plant in Kisii county and Kabarnet Level V Hospital (0^{0}489030 N, 035.741070 E) in Baringo County Kenya in the months of June and December to correspond with dry and wet seasons witnessed in Kenya in 2020. 8 grab samples per site were done each comprising of 4 influent samples, and 4 effluent samples both during the wet and dry seasons, using polypropylene bottles (1L) pre-rinsed with ultrapure water. The collected samples were then preserved in a cool box at 4 °C until arrival to the laboratory for analysis.

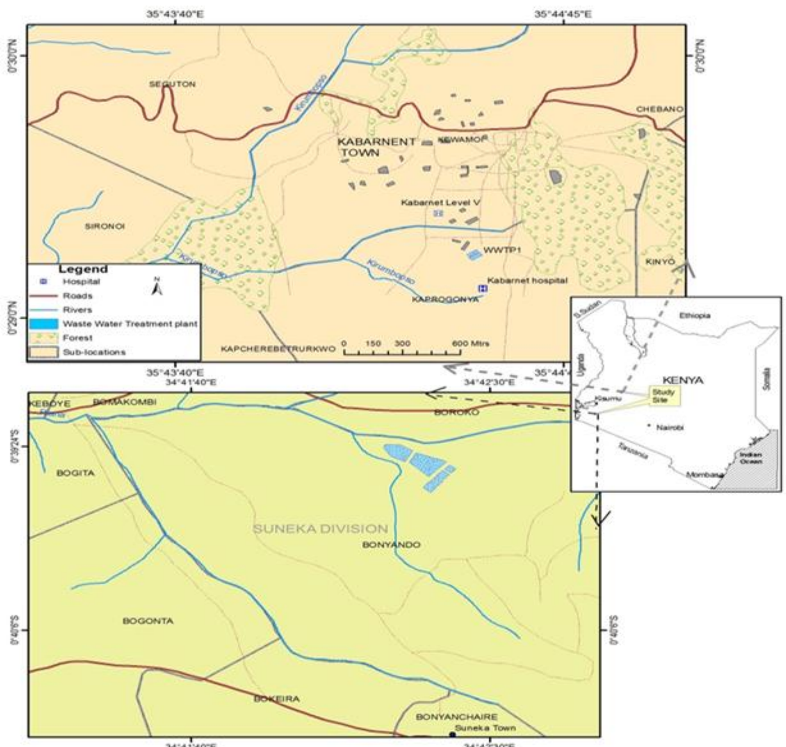

Figure 1: Shows the Map of Kenya and the two sampling sites

2.3 Sample extraction and storage.

The waste water samples were first centrifuged at 4000 rpm for 40 minutes and subsequently filtered through a 2.7 µm GF/D Whatmann glass microfiber filter papers then through a 1.0 µm GF/B Whatmann glass microfiber filter, and lastly through a 0.45 µmWhatmann nylon filter (VWR, Belgium) under vacuum (Fioroni Filters, Ingré, France). The filtered samples were the collected in dried pre-cleaned glass reservoirs. Sample storage and handling in the laboratory was done based on the methods described byV. Diwan *et al*[12] with little modifications. MCX SPE catridges were first conditioned using 3mL ethyl acetate; then 3ml methanol (HPLC grade) and finally 3ml HPLC grade water at a flow rate of 3mL min^{-1}.After loading, the cartridges were then washed with a volume of 3mL HPLC grade water, elution was performed using 10 mL of HPLC-grade methanol, and then evaporated to dryness under vacuum conditions. The residue analytes were re-dissolved to 2 mL with HPLC-grade methanol. All samples were filtered using 0.45-µm nylon micro filters prior to injection to LC instrument [13].

2.4 Antibiotics quantification

Antibiotics concentrations and quantification were done using methods developed and validated as per the International Conference on Harmonization ICH guidelines [14]. An Agilent 1220 series with a UV/Vis detector coupled with a Phenomenex RP- (C_{18}; 4.6mm i.d. x 250mm x 5µm) column was used for the separation and quantification of analytes.

The column temperature was set at $45^{0}C$. The mobile phase consisted of Acetonitrile: Methanol: 0.1% aqueous formic acid (65:30:5) with a flow rate of rate – 0.75mL/min. The optimum flow rate was then investigated by interchanging the flow rates from 1 mL.min-1, 0.8 mL.min-1, 0.6 mL.min-1 and 0.4 mL.min-1.

Two pressure levels were evaluated for pump pressure A and pump pressure B set at a maximum of 15.0 MPa and a minimum of 2.0 MPa. Two sets of injection volumes 10 µL and

20 µL of sample were also compared to get the best peak resolution. The chromatic conditions of each antibiotics sample are appended in table 1 below

Table 1: Chromatographic conditions of each individual antibiotic

Antibiotics	Injection volumes	Flow rate	Column Temperature (^{0}c)	Wavelength (nm))
AMP	10µl.	0.75mL/min	45	320
AMX	10µl.	0.75mL/min	45	334
TRI	10µl.	0.75mL/min	45	237
SMZ	10µl.	0.75mL/min	45	259

The XCalibur v1.4 software was used to integrate the peak areas with analyte identification based on the comparisons of standards to those of the unknown. Periodically both blanks and standards were run to ensure quality assurance.Method validation, was done by periodically spiking the samples and blank water samples with mixed standard solutions at the concentrations range of between 1.5 to 48µg/L. Each method was evaluated for their limits of detections (LOD) and limits of quantification (LOD) based on their standard deviations. The Limits of detections were calculated at 3.3 σ/s while the Limits of quantifications were calculated at 10 σ/s via σ of the spiked sample solutions respectively. The methods linearity were validated by use of regression coefficient (R^2), while the methods robustness was analyzed by intentional variations in flow rates and pump pressures.

2.5 Removal efficiencies

Removal efficiencies of the two WWTP for the selected antibiotics were calculated using the concentrations quantified at the influents and effluents as per equation 1 below [15].

Removal Efficiency (%) = $\frac{C_{Influent} - C_{Effluent}}{C_{Influent}}\ \mathbf{x\ 100}$ Equation 1

3. RESULTS AND DISCUSSION

The calibration curves that were used for the quantification of antibiotics all had a goodlinearity with an R^2 value greater than 0.98 for all the antibiotics. The LOD for the antibitics, ranged from 0.293 upto 1.083 µg/l while the the LOQ ranged from 0.887 to 3.28 µg/l. as shown in table 2.

Table 2: Percentage recoveries and other calibration parameters

Compound	% Recovery	R^2	LOD in µg/l	LOQ in µg/l
Ampicillin	98	0.9992	0.526	1.59
Amoxicillin	92	0.9983	1.084	3.28
Trimethoprim	89	0.9881	0.293,	0.887
Sulfamethoxazole	92	0.9997	0.3288	0.9965

3.1 Occurrence of selected antibiotics in WWTP1 and WWTP2

The results indicated that all the four antibiotics were detected at the influent and effluents of both the sample study sites in the wet and dry seasons. Table 3 below, indicates the mean levels of antibiotics in raw influent and effluent of both Kisii Waste water (WWTP1) treatment plant and Kabarnet wastewater treatment plant (WWTP2).

Table 3: Mean distribution of selected antibiotics in dry and wet seasons in WWTP1 and WWTP2

Groups	Sample	Samples	WWTP1 M± SD(µg/l)		WWTP 2 Mean± SD (µg/l)	
			Wet	Dry	Wet	Dry
Sulfonamides	SMX	Influent	1.11 ±0.01	1.29±.0.07	0.14±0.02	0.18±0.01
	SMX	Effluent	0.15 ±0.03	0.16±0.03	0.13 ±0.01	0.14±0.02
	TRI	Influent	0.14 ±0.0	0.09±0.01	0.06 ±0.010.05±0.01	
	TRI	Effluent	0.07±0.0	0.069±0.03	0.03 ±0.010.02±0.00	
Penicillin	AMP	Influent	2.04±0.2	2.92±0.32	1.01 ±0.011.34±0.03	
	AMP	Effluent	0.69±0.0	0.81±0.06	0.22 ±0.000.09±0.0	
	AMX	Influent	1.34±0.01.81±0.03		0.09 ±0.010.09±0.02	
	AMX	Effluent	0.08±0.000.54±0.02		0.07 ± 0.010.08±0.00	

The results showed that all the antibiotics investigated in this study, namely amoxicillin, Ampicillin, Trimethoprim and Sulphamethaxazole were present in both waste water treatment plants at both the influent and the effluent in the dry and wet seasons. The Kisii waste water treatment had higher levels of antibiotics maybe due to the fact that the Kisii waste water treatment plant serves a larger population that the Kabarnet waste water treatment plant as suggested by [16].

3.2 Removal efficiencies

The removal efficiencies of WWTP 1 and WWTP2 were calculated and the results tabulated in table 3 below. The highest percentage removal was for amoxicillin which was approximately 94.3 %. This results are inline with other results that have been reported globally which suggest that most conventional waste water treatment plants are inefficient in the removal of antibiotics from waste water [17], [18].

Table 4: Percentageremoval efficiencies of antibiotics in dry and wet seasons in WWTP1 andWWTP2

Groups	Sample	WWTP1 %removal		WWTP 2 % removal	
		Wet	Dry	Wet	Dry
Sulfonamides	SMX	86.48	87.57	7.14	22.22
	TRI	50	23.33	50.00	40.00
Penicillin	AMP	66.17	72.26	78.21	93.28
	AMX	94.03	70.17	22.22	11.11

4. CONCLUSION

Amoxicillin, ampicillin, trimethoprim and sulphamethaxazole were found in both Kisii WWTPs and Kabarnet WWTPs with nearly all antibiotics having a higher concentration of in the dry season than in the wet season. The highest concentrations detected for antibiotics were for amoxicillin at 2.92 µg/l while the lowest concentrations detected were for Trimethoprim at 0.02 µg/l. The highest calculated percentage removal was 94.03 % while the lowest calculated percentage removal was 7.14 %. The results suggest that the two WWTPs are not sufficient for the removal of different types of antibiotics. It is therefore recommended that more advanced systems should be adopted to reduce the amounts of antibiotics in waterbodies

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