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Title: Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments

Year: 2020

Version: Accepted version (Final draft)

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Please cite the original version:

Kairigo, P., Ngumba, E., Sundberg, L.-R., Gachanja, A., & Tuhkanen, T. (2020). Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. *Science of the Total Environment*, 720, Article 137580.

<https://doi.org/10.1016/j.scitotenv.2020.137580>

13

ABSTRACT

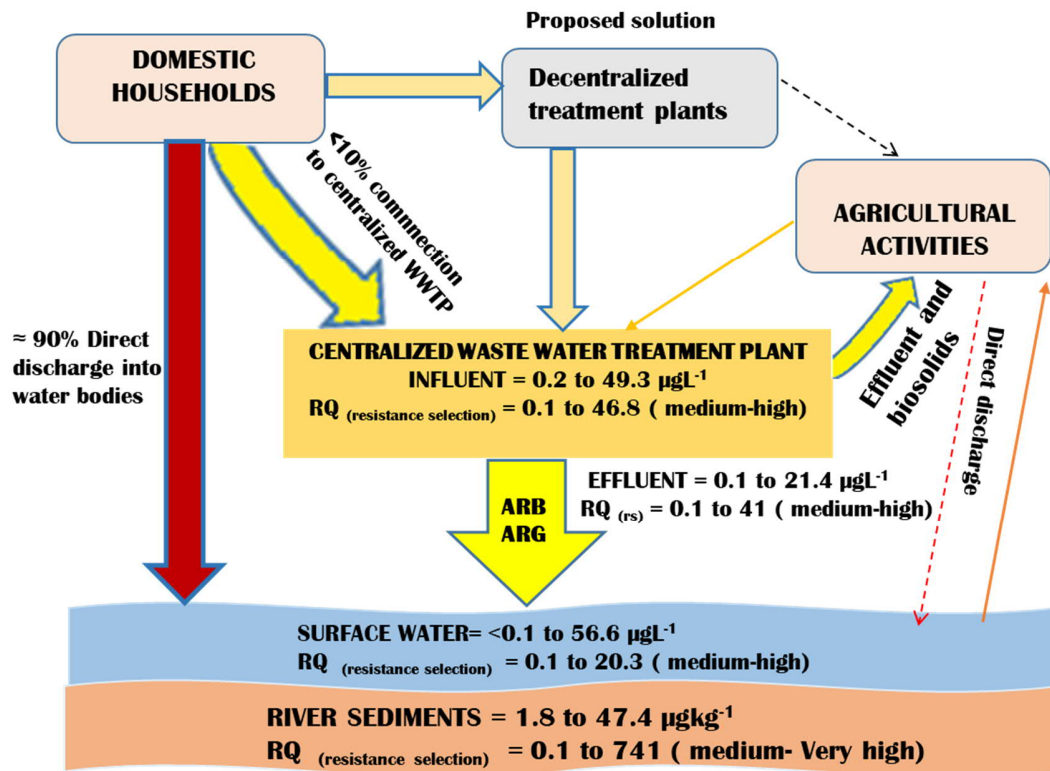
14 Active pharmaceutical ingredients, especially antibiotics, are micropollutants whose continuous
15 flow into hydrological cycles has the potential to mediate antibiotic resistance in the environment
16 and cause toxicity to sensitive organisms. Here, we investigated the levels of selected antibiotics
17 in four wastewater treatment plants and the receiving water bodies. The measured environmental
18 concentrations were compared with the proposed compound-specific predicted no-effect
19 concentration for resistance selection values. The concentration of doxycycline, amoxicillin,
20 sulfamethoxazole, trimethoprim, ciprofloxacin and norfloxacin within the influents, effluents,
21 surface waters and river sediments ranged between 0.2 and 49.3 μgL^{-1} , 0.1 to 21.4 μgL^{-1} ; < 0.1
22 and 56.6 μgL^{-1} ; and 1.8 and 47.4 μgkg^{-1} , respectively. Compared to the effluent concentrations,
23 the surface waters upstream and downstream one of the four studied treatment plants showed two
24 to five times higher concentrations of ciprofloxacin, norfloxacin and sulfamethoxazole. The risk
25 quotient for bacterial resistance selection in effluent and surface water ranged between <0.1 and
26 53, indicating a medium to high risk of antibiotic resistance developing within the study areas.
27 Therefore, risk mitigation and prevention strategies are a matter of priority in the affected areas.

28 **Keywords**

29 Antibiotics; wastewater; antimicrobial resistance; antibiotic resistance evolution, risk assessment.

30

31 **Graphical Abstract**



32

33 **Highlights**

- 34 ➤ Direct discharge of wastewater contribute to flux of antibiotics in rivers
- 35 ➤ zero or negative pharmaceutical removal efficiencies measured in WWTP's
- 36 ➤ Residual antibiotics occurred above predicted no-effect concentrations
- 37 ➤ Decentralized sanitation solutions are proposed for risk control
- 38 ➤ Risk of evolution of antibiotic resistance greatest in wastewater and river sediments

39 **1. Introduction**

40 Consumption of antibiotics has increased globally (Klein et al., 2018). This is due to increased
41 disease burden, increased availability, especially of over-the-counter prescriptions, and increased
42 resistance of pathogenic bacteria to the available antimicrobial agents (Gelband et al., 2015; Klein
43 et al., 2018; Van Boeckel et al., 2014). The occurrence, fate and removal of active pharmaceutical
44 ingredients (APIs), especially antibiotics, in hydrological cycles is an environmental pollution
45 issue of global concern (aus der Beek et al., 2016; Daughton, 2016). The presence of
46 pharmaceuticals in aquatic environments is especially high in developing countries. Studies across
47 Africa have reported varying concentrations of common antibiotics ranging from ngL^{-1} to several
48 orders of magnitude higher. According to a global review of API prevalence in the hydrological
49 cycles, Europe and North America indicated relatively low prevalence (aus der Beek et al., 2016)
50 compared with many developing countries, especially in Africa (Madikizela et al., 2017). In
51 Kenya, APIs has been assessed in only a few studies, covering the Nairobi river basin, Nzoia river
52 basin and Kisumu (K'oreje et al., 2018, 2016; Ngumba et al., 2016). However, the prevalence of
53 environmental residual antibiotics in most parts of the country remains unknown. High population
54 densities in urban and peri-urban areas, characterized by informal settlements, lack of proper
55 sanitation facilities and high prevalence of disease (especially tuberculosis and HIV/AIDS)
56 indicate the need to systematically assess the presence of pharmaceuticals in the environment.

57 The majority of pharmaceuticals do not metabolize completely and therefore are excreted into the
58 environment either in their original form or as pharmacologically active metabolites or
59 transformational products (Carvalho and Santos., 2016a). Depending on the category of the
60 compound, 50–90% of ingested APIs are excreted through urine (Kümmerer, 2009; Tran et al.,
61 2016). These APIs and their active metabolites flow into the hydrological cycles by direct

62 discharge into the environment or through wastewater treatment plants (Kümmerer, 2008; Luo et
63 al., 2014; Matongo et al., 2015; Zhang et al., 2015).

64 The presence of antibiotics in the environment at levels below therapeutic concentration may
65 catalyze the ability of bacteria to develop antibiotic resistance (Kümmerer, 2003). As such, an
66 environmental concentration of antimicrobials at subinhibitory levels favors the growth of both
67 resistant and susceptible bacterial genotypes (Khan et al., 2017). These lower concentrations give
68 competitive advantage to the growth of resistant strains (Andersson and Hughes, 2014). This may
69 lead to the selection of highly resistant bacteria which present a greater management challenge (Li
70 et al., 2016). Antimicrobial resistance (AMR) in wastewater, surface and treated water has been
71 reported in various studies (Prestinaci et al., 2015; Sabri et al., 2018; Sobsey et al., 2014). The
72 World Health Organization (WHO) has previously pronounced AMR a threat to global health
73 (WHO, 2016).

74 This study was undertaken to determine the prevalence of selected antibiotic residues in the
75 wastewater, surface water and river sediments from three counties in Kenya. Environmental
76 sample processing and trace level analysis was carried out using a liquid chromatography
77 electrospray ionization tandem mass spectrometer (LC-ESI-MS/MS) according to methods
78 published by Ngumba et al. (2016) for liquid samples and Al-Khazrajy and Alistair (2017) for
79 river sediments. Furthermore, we carried out risk assessments for resistance selection, based on
80 the compound-specific predicted no-effect concentrations (PNEC) for resistance selection values
81 proposed by Bengtsson-Palme and Larsson (2016). The PNEC for resistance selection was
82 calculated based on the European Committee for Antimicrobial Susceptibility Testing (EUCAST)
83 database for multiple genera and families of pathogenic microorganisms.

84 **2. Materials and methods**

85 **2.1 Study area and sample collection**

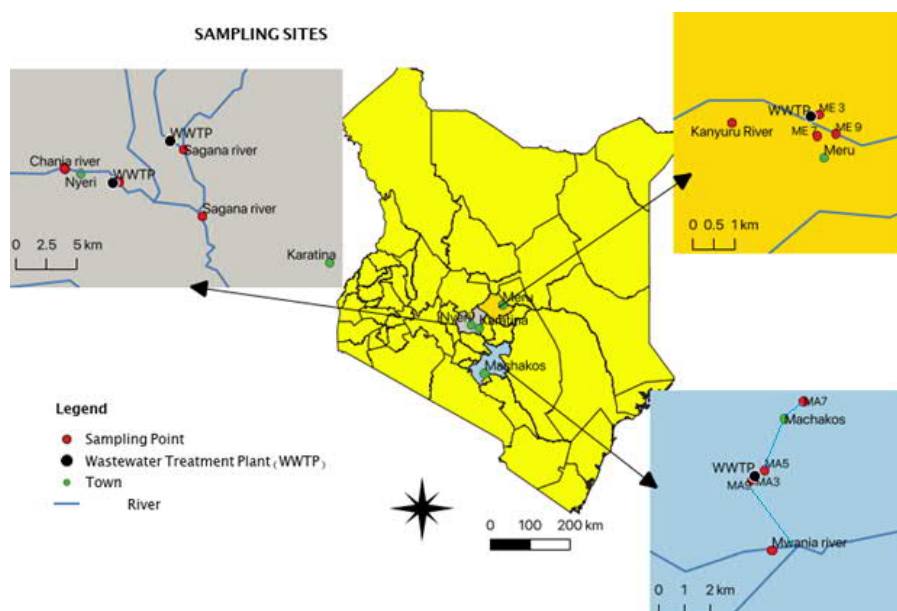
86 A five-day sampling campaign was carried out in the administrative towns of the counties of
87 Machakos, Nyeri and Meru in the Republic of Kenya. A total of four wastewater treatment plants
88 were sampled altogether: three wastewater stabilization ponds Machakos (WWTP1), Gateei in
89 Nyeri (WWTP 2), Meru (WWTP 4), and one trickling filter treatment plant in Kangemi (WWTP
90 3) Nyeri County. Machakos County is situated 80 km southeast of Nairobi while Nyeri and Meru
91 counties are located in the Mount Kenya region, approximately 150 km and 250 km north of the
92 capital city, Nairobi. The selected sampling area demographics are shown in Table 1. Currently,
93 the actual number of inhabitants served by these treatment plants cannot be accurately estimated
94 due to the various informal settlements mushrooming within the vicinity of the sewer line and the
95 illegal connections to it. Furthermore, wastewater soak pits, septic tanks and pit latrines are
96 frequently found in these areas. Sampling coordinates for all the sampling spots are provided in
97 Table S1 in the supplementary information.

98 **Table 1:** The population, percentage access to improved sources of water, sanitation and
99 sewerage system for the selected sampling areas according to the Kenya National Bureau of
100 Statistics (2013).

Location	Population	Access to improved sources of water (%)	Access to improved sanitation (%)	Access to sewerage system (%)
Machakos town	195,029	42.2	61	5.7
Nyeri town	111,656	85	66	16.5
Meru town	144,275	79.5	92.2	3.4

101

102 Sampling was done in January 2019, which is usually a dry month preceding short rains. Four
103 different waste water treatment plants (WWTPs) and the rivers to which they discharge were
104 sampled, as shown in Figure 1. Hourly 1L grab samples were collected from the WWTPs influent
105 and effluent over a period of 8 h with 60 min interval and samples pooled to get representative 1
106 L composite samples. Duplicate 1L river water samples were collected approximately 200 m and
107 2 km upstream and downstream of the effluent discharge points. Sediment samples were also
108 collected at a depth of approximately 5 cm from all water sampling points and air dried indoors at
109 room temperature (25 °C).



110
111 **Figure 1:** The map of Kenya and the extrapolated sampling sites in Machakos, Meru and Nyeri

112 *2.2 Chemicals and standards*

113 The pharmaceutical standards used were of >99% purity and obtained from Sigma Aldrich (US).
114 The physicochemical properties of the standards, including their structure and CAS registry
115 numbers, are indicated in Table S2 of the supplementary information. All the isotopically labeled
116 internal standards were purchased from Alsachim (France) apart from [²H₉]-TMP which was

117 purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol
118 were purchased from Merck (Germany), ammonium hydroxide (25%) solution was purchased
119 from Merck (Belgium), formic acid and formic acid (98%) from Fluka (Germany). Stock solutions
120 were prepared as outlined by Ngumba et al. (2016) and stored at +4 °C in amber vials.

121 **2.3 Sample extraction**

122 200 mL duplicate sub-samples were measured from the pooled sample and 40 μL of 10 mgL^{-1}
123 isotopically labelled mixed standard was added to each before processing. Samples were filtered
124 through a 47mm GF/F ($0.7\ \mu\text{m}$) glass filter followed by solid-phase extraction using Oasis HLB
125 6 cc (200 mg) cartridges. The extraction and analytical method developed by Ngumba et al. (2016)
126 was used for the liquid samples. The sample concentration of the target compounds doxycycline
127 (DOX), amoxicillin (AMO), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP)
128 and norfloxacin (NOR) was measured. Target compounds were extracted from the sediment
129 samples using an ultrasonic bath. As outlined by Al-Khazrajy and Alistair (2017), 5 g of the air-
130 dried sediment samples was extracted and the extracts subjected to the solid-phase extraction
131 process. In brief, the HLB cartridges were conditioned with 6 mL of methanol followed by 6 mL
132 of Milli-Q ultrapure water at a flow rate of $5\text{ mL}/\text{min}^{-1}$. Samples spiked with isotopically labelled
133 internal standards were loaded at the same flow rate, after which the target compounds of interest
134 were eluted with 4 mL of 50:50 acetonitrile-methanol solution. The eluting solvent was evaporated
135 under a stream of N_2 gas at $40\text{ }^\circ\text{C}$ and the sample reconstituted to 1 mL using 20:80 ACN:H₂O
136 solvent. Filtration was done through a $0.2\ \mu\text{m}$ cellulose acetate membrane filter into HPLC vials
137 ready for analysis.

138 **2.4 LC-ESI-MS/ MS analysis**

139 A waters alliance 2975 liquid chromatograph (LC, Milford, MA, USA) was used for separation.
140 An Xbridge™ (3.5 µm x 2.1 mm x 100 mm) C₁₈ reversed-phase column fitted with a Vanguard®
141 (2.1mm x 5mm) pre-column was used. A Quattro micro mass spectrometer (MS) was used for
142 detection. The LC solvent systems and the MS/MS instrument parameters optimized by Ngumba
143 et al. (2016) were used for the targeted multiresidue analysis. The optimized LC-ESI-MS/MS
144 instrument parameters for the analysis of the target compounds are shown in Table S3 of the
145 supplementary information. Figures S1 and S2 of the supplementary information show the internal
146 standard calibration and the matrix matched calibration graphs, respectively.

147 **2.5 Removal efficiencies**

148 The percentage removal efficiency (RE %) of the selected APIs from the WWTP was evaluated
149 using Equation 1.

150
$$RE (\%) = \frac{(C_{Inf} - C_{Eff})}{C_{Inf}} * 100 \quad \text{Equation (1)}$$

151 Where C_{Inf} and C_{Eff} refer to the respective measured concentrations (µgL⁻¹) at the influent and
152 effluent of the WWTP (Sun et al., 2015).

153 **2.6 Risk assessment of antimicrobial resistance selection**

154 The risk quotient (RQ) for antimicrobial resistance selection within the sampled environments was
155 indirectly determined (Tran et al., 2019), according to the measured residual antibiotic
156 concentrations in the representative water samples and the predicted no-effect concentration
157 (PNEC) for resistance selection (RS) as illustrated in Equation 2. The compound-specific
158 PNEC_(RS) values used for risk assessment were proposed by Bengtsson-Palme and Larsson (2016)

159 based on the EUCAST database. The $PNEC_{(RS)}$ values also factored multiple genera of pathogenic
160 microorganisms present in the environment.

$$161 \quad RQ = \frac{MEC}{PNEC_{(RS)}} \quad \text{Equation (2)}$$

162 MEC is the measured environmental concentration in the representative samples and $PNEC_{(RS)}$ is
163 the compound-specific predicted no-effect concentration for resistance selection as proposed by
164 Bengsston-Palme and Larsson (2016). The RQ results were classified as low, medium and high
165 risk and the interpretation followed the format $RQ \geq 1$ for high risk, $1 > RQ \leq 0.1$ for medium risk
166 and $RQ < 0.1$ for low risk (Abafe et al., 2018; Guo et al., 2016; Hanna et al., 2018).

167 **3. Results**

168 **3.1 LC-ESI-MS/MS analysis**

169 The results of the LC-ESI-MS/MS analysis are illustrated in Table 2. The linear correlation
170 coefficient (r^2) values of the calibration curves was > 0.99 for all the target compounds. The limit
171 of detection (LOD) and limit of quantification (LOQ) values varied relatively across the analytes
172 with the majority having an $LOQ \leq 10 \text{ ngL}^{-1}$. DOX had the highest LOQ value of 135 ngL^{-1} .

173 **Table 2:** LC-ESI-MS/MS Method qualification results

Compound	RT (SD)	r^2	LOD (ngL^{-1})	LOQ (ngL^{-1})
AMO	1.77(0)	0.994	8	22
CIP	2.24 (0.08)	0.99	3	10
TMP	2.25 (0.05)	0.999	3	7
NOR	2.15 (0.06)	0.994	4	8
SMX	4.83 (0.02)	0.996	7	18
DOX	5.87 (0.01)	0.994	56	135

174

175 **3.2 Prevalence of antibiotics and removal efficiency**

176 The concentration of the targeted antibiotics in the wastewater influents, effluents, surface waters
 177 and river sediments as well as the corresponding percentage removal are shown in Table 3. The
 178 standard deviation of the measurements is shown in parenthesis. SMX was the most abundant
 179 antibiotic in all the sampling sites with values ranging from 0.03(0.01) μgL^{-1} to 56.6(4.0) μgL^{-1} .
 180 The highest value was measured in the surface water grab sample MA7, sampled approximately
 181 200 m downstream of the effluent discharge point of WWTP1. AMO, which is a common
 182 aminopenicillin beta-lactam antibiotic, occurred at concentrations of 0.9(0.1), 0.05(0.01) and
 183 0.3(0.1) μgL^{-1} in surface water samples MA7, MA8 and NY9, respectively. These were relatively
 184 low levels compared with the corresponding river sediment phase, in which concentrations of
 185 4.6(0.3), 43.8(3.1), 11.7(3.2) and 7.8(1.6) μgkg^{-1} were measured for samples MA9, NY10, ME6
 186 and ME10, respectively.

187 **Table 3:** concentrations (μgL^{-1}) of the selected antibiotics in the sampled WWTP's. AMO =
 188 Amoxicillin, CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX =
 189 Sulfamethoxazole, and DOX = Doxycycline

	Site	Sample type	Code	AMO	CIP	TMP	NOR	SMX	DOX
Machakos	WWTP 1	Influent	MA 1	4.6(0.2) ¹	1.6(0.4)	5.6(0.1)	1.2(0.1)	49.3(2.7)	2.7(0.2)
		Effluent	MA 3	1.6(0.3)	0.4(0.3)	0.3(0.2)	0.5(0.2)	8.5(0.4)	1.5(0.4)
	Mitheu River	Surface water grab (200m upstream)	MA 4	<LOQ	1.3(0.1)	<LOQ	0.6(0.01)	49.7(1.5)	0.7(0.1)
		river sediment (200m upstream)	MA 5	n.d ³	29.3(7.2)	<LOQ	<LOQ	<LOQ	<LOQ
		Surface grab (2 km upstream)	MA 6	<LOQ	0.7(0.1)	0.2(0.1)	0.9(0.3)	0.06(0.02)	<LOQ
		Surface water grab (200m downstream)	MA 7	0.9(0.01)	0.5(0.1)	0.1(0.03)	2.2(0.4)	56.6(4.4)	<LOQ
	Mwania river	Surface grab (2 km downstream)	MA 8	0.05(0.01)	0.5(0.1)	<LOQ	0.11(0.01)	1.2(0.1)	0.3(0.1)

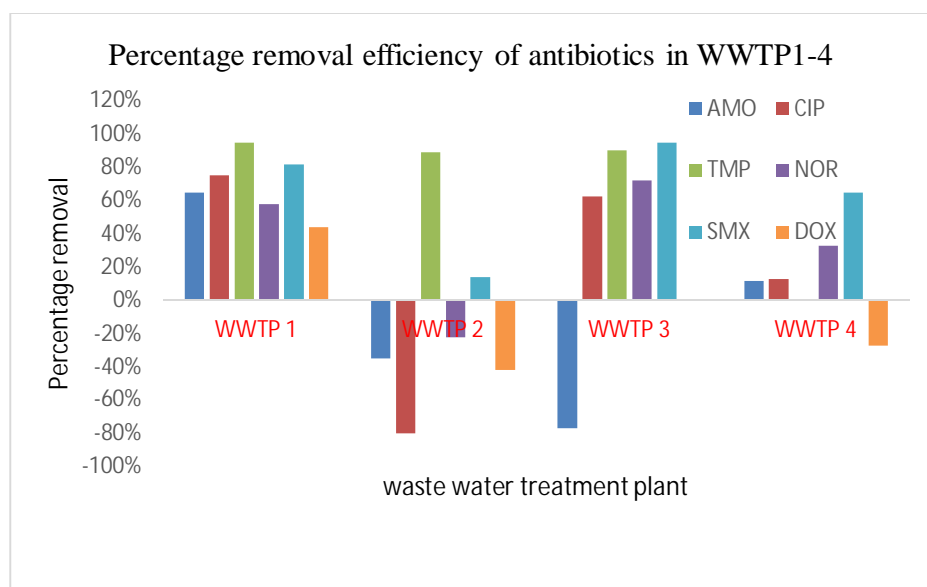
		River sediment (2 km downstream)	MA 9	4.6(0.3)	<LOQ	<LOQ	<LOQ	3.4(0.7)	8.2(1.3)	
Nyeri county	Gatei WWTP 2	Influent	NY 1	0.2(0.06)	<LOQ	0.9(1.8)	0.9(0.1)	24.9(1.7)	<LOQ	
		Effluent	NY 3	0.9(0.2)	1.8(0.2)	0.1(0.01)	2.9(0.1)	21.4(3.4)	0.7(0.01)	
	Sagana river	Surface water (1km downstream)	NY 4	n.d	0.2(0.1)	<LOQ	<LOQ	n.d	n.d	
		river sediment	NY 5	n.d	n.d	n.d	n.d	n.d	n.d	
	Kangemi WWTP 3	Influent	NY 6	0.7(0.2)	0.8(0.1)	4.8(0.3)	2.8(0.1)	25.47(1.8)	0.4(0.1)	
		Effluent	NY 8	1.24(0.3)	0.3(0.1)	0.5(0.1)	0.8(0.3)	1.3(0.4)	0.4(0.1)	
		Chania river	Surface water (2m downstream)	NY 9	0.3(0.1)	n.d	<LOQ	0.1(0.03)	0.3(0.05)	n.d
			river sediment(2m downstream)	NY 10	43.8(3.1)	n.d	<LOQ	26.0(3.8)	16.3(3.9)	<LOQ
			Surface water (5m upstream)	NY 11	<LOQ	<LOQ	<LOQ	<LOQ	n.d	n.d
			river sediment (5m upstream)	NY 12	5.9(1.4)	<LOQ	1.8(0.5)	26.6(3.8)	<LOQ	32.2(5.7)
		Surface water (200m)	NY 13	n.d	<LOQ	<LOQ	0.1(0.4)	n.d	<LOQ	
		river sediment	NY 14	<LOQ	35.7(4.2)	13.3(2.5)	6.6(1.4)	<LOQ	7.8(2)	
	Meru County	WWTP 4	Influent	ME 1	1.58(0.1)	3.0(0.7)	0.1(0.01)	1.2(0.3)	49.1(5.1)	<LOQ
			Effluent	ME 3	1.4(0.1)	2.6(0.4)	0.1(0.04)	0.8(0.1)	17(1.7)	0.5(0.1)
Kanyuru River		river source swamp (2km upstream)	ME 5	<LOQ	0.24	n.d	n.d	<LOQ	0.1(0.01)	
		river sediment (2km upstream)	ME 6	11.7(3.2)	47.4(2.8)	<LOQ	<LOQ	44.7(3.9)	<LOQ	
		surface grab (500m downstream)	ME 7	n.d	0.2(0.03)	<LOQ	<LOQ	n.d	0.02(0.01)	
		river sediment (500m downstream)	ME 8	<LOQ	<LOQ	<LOQ	<LOQ	10.4(1.3)	13.9(2.4)	
		Surface grab (1km downstream)	ME 9	n.d	0.2(0.05)	<LOQ	<LOQ	n.d	0.1(0.03)	
		river sediment(1km downstream)	ME 10	7.8(1.6)	<LOQ	<LOQ	<LOQ	<LOQ	11.4(2.1)	

190 ¹Concentration of the analytes reported in μgL^{-1} () standard deviation, n=2, ² <LOQ - Below Quantification limit ³ n.d - not
191 detected/below limit of detection

192 The prevalence of the selected antibiotics was higher in the samples taken from the river sediments
193 than in those from the surface waters. River sediment sample MA9 had SMX, AMO and DOX
194 concentrations of 3.4(0.7), 4.6(0.3) and 8.2(1.3) μgkg^{-1} , respectively, which were considerably
195 higher than the values of 1.2(0.1), 0.05(0.01) and 0.3(0.1) μgL^{-1} found in the surface water sampled

196 at the same location. Similar trend in phase distribution of the antibiotics was recorded in sediment
 197 samples NY10, NY12 and NY14 with the following concentration ranges: AMO, 5.9(1.3) to
 198 43.8(3.1); CIP, < LOQ to 35.7(4.2); NOR, 6.6(1.4) to 26.6(3.8); DOX, 7.8(2) to 32.2(5.7) μgkg^{-1} .
 199 Sediment sample ME6, which was collected upstream of WWTP4, had higher concentration of
 200 AMO, CIP and SMX compared with downstream samples from the same site.

201 The removal efficiency of specific compounds at the WWTPs varied between 0 and 95%.
 202 However, higher concentrations in the effluent relative to the influent, which accounted for the
 203 negative removal efficiencies, was noted especially for AMO, CIP, NOR and DOX, as shown in
 204 Figure 2



205
 206 **Figure 2:** Percentage removal efficiencies of the antibiotics in the selected treatment plants

207 Generally, these findings provide evidence of environmental concentrations of residual antibiotics
 208 above their respective PNECs for resistance selection. This could signal the ineffectiveness of the
 209 existing wastewater treatment plants in removing APIs.

210 **3.3 Risk assessment of antibiotics for resistance selection**

211 The RQ for antibiotic resistance selection, calculated based on the compound-specific $PNEC_{(RS)}$
 212 values as proposed by Bengtsson-Palme and Larsson (2016) are shown in Table 5. The risk of
 213 resistance selection in the aqueous phases ranged between medium and high. The high-risk figures
 214 were for AMO, NOR and CIP, with RQ values of 6.4, 5.8 and 41, respectively. Wastewater
 215 samples carried a higher risk than surface water samples, except for SMX, which exhibited a higher
 216 risk in surface water. The same compounds accounted for the increased risk of resistance selection
 217 assessed in the sediment phase. Resistance selection was one to two times more likely to occur in
 218 the wastewater and river sediment phases than in the surface water.

219 **Table 5:** Concentrations and risk quotient for resistance selection for the selected antibiotics in
 220 Kenya. MEC=Measured Environmental Concentrations, PNEC=Predicted no effect concentration
 221 for resistance selection as proposed by Bengtsson-Palme and Larsson (2016). AMO = Amoxicillin,
 222 CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and
 223 DOX = Doxycycline.

API	MEC (μgL^{-1}) This study		PNEC (RS) (μgL^{-1}) ^a (resistance selection)	Covered genera ^b (families)	Risk quotient (resistance selection)
	Effluent	surface water			
AMO	0.9-1.6	0.05-0.9	0.25	19(12)	0.2-6.4 (medium - high)
NOR	0.5-2.9	0.1-2.2	0.5	12(8)	0.2-5.8 (medium -high)
TMP	0.1-0.5	0.1-0.2	0.5	15(7)	0.2 -1 (medium - high)
CIP	0.43-2.6	0.2-1.3	0.064	29(18)	3.1 - 40.6 (high)
SMX	1.3-21.4	0.1-56.6	16	6(4)	<0.1 - 3.53 (low-high)
DOX	0.4-1.5	0.1-0.7	2	20(11)	<0.1 - 0.7 (low-medium)

^aPNEC value corresponds to the size-adjusted lowest MIC divided by an assessment factor of 10 as proposed by Bengtson-palme and Larsson (2016).

^bThe number of different bacterial genera and families tested against the specific antibiotic.

224

225 **4. Discussion**

226 *4.1 fate of antibiotics in the natural environment*

227 The MEC for the analyzed antibiotics mostly occurred in low concentrations in the aqueous
228 samples as compared with the sediment samples. The low levels of AMO in the aqueous phase
229 may be attributed to the fact that beta-lactams are relatively hydrophobic, tend to migrate to the
230 sediment phase and are generally highly susceptible to hydrolysis either by chemical or enzymatic
231 agents (Hirte et al., 2016).

232 TMP was mostly detected in wastewater influent and river sediment, but infrequently in the surface
233 water. TMP-SMX combinations are used to treat broad spectrum infections including cholera.
234 They are also administered to immunosuppressed patients as prophylaxis against opportunistic
235 infections (Kronbichler et al., 2018; Walker et al., 2010).

236 At one of the four sampling sites, the concentration of target compounds in river water upstream
237 and downstream of this treatment plant (WWTP1) was considerably higher than the concentration
238 in the influent and effluent. This could be attributed to direct discharge of untreated wastewater
239 into water bodies, taking into account that less than 10% of the population in these areas are
240 connected to the centralized sewage treatment system. Intentional tampering and blockage of the
241 sewer line en route to the plant was noted. This was done to divert the sewerage water into the
242 river for vegetable farming along the river banks. It is highly likely that this directly contributed

243 to the higher levels of the pharmaceutical compounds in the river samples compared to those found
244 in the plant effluent samples.

245 The concentration levels determined in the sediment samples indicated accumulation of some of
246 the antibiotics, mostly SMX and DOX, in the sediment phase as compared to the aqueous phase.
247 The higher measured concentrations in the sediment samples could mean that residual antibiotics
248 exert higher selection pressure within the sediment phase than in the aqueous phase.

249 The irregular flux in environmental concentrations of antibiotics between the influent, effluent,
250 surface water and river sediments could be attributed to hydrological flow conditions. During dry
251 seasons, the concentration could be higher, and vice versa for wet seasons due to dilution. This
252 automatically influences chemical and biological reactions within the natural environment. Waste
253 stabilization ponds, such as those sampled in this study, have limited ability to remove recalcitrant
254 organic matter (Ignatev and Tuhkanen, 2019). Accumulation could be the result of the sludge being
255 removed with irregular frequency, as well as the resuspension of the adsorbed APIs in the sludge,
256 especially when decomposition occurs in well aerated conditions (Ho et al., 2017).

257 Negative removal efficiencies for APIs have been reported (K'oreje et al., 2018; Li et al., 2009;
258 Polesel et al., 2016; Thiebault et al., 2017; Udert et al., 2015). Factors causing this may include
259 elimination of antibiotics adsorbed into the particulate matter during sample processing and
260 unaccounted-for hydraulic retention time during sampling. Physicochemical changes during the
261 treatment process influence the adsorption behavior of the antibiotics and hence affect the partition
262 ratio between the aqueous, suspended and sediment phases, and between the influent and effluent
263 concentration (Lindberg et al., 2005). API accumulation, biotic or abiotic dissolution, as well as
264 back transformation and de-conjugation of metabolic products back to parent compounds, can all
265 lead to increased measured concentrations in the effluent relative to the influent (Archer et al.,

266 2017; Haddad et al., 2015; Polesel et al., 2016). SMX transformational products have been shown
 267 to back transform to the parent compound under biological and photolytic degradation conditions
 268 (Archer et al., 2017; Bagnis et al., 2020). Previous studies in Kenya have reported the presence of
 269 14-112 μgL^{-1} of SMX and 4-20 μgL^{-1} of TMP in wastewater influent, and 10 μgL^{-1} of SMX in the
 270 effluent (K'oreje et al., 2018). In addition, two independent studies of the Nairobi river surface
 271 water reported SMX concentrations of 13.76 μgL^{-1} (Ngumba et al., 2016) and 23.35 μgL^{-1} (K'oreje
 272 et al., 2012) and TMP concentrations 2.65 μgL^{-1} (Ngumba et al., 2016) and 9.48 μgL^{-1} (K'oreje et
 273 al., 2012), respectively. In this article, we report values of the same order of magnitude as other
 274 Kenyan studies, but considerably higher than those reported in the global North, as shown in Table
 275 4.

276 **Table 4.** Previous observations of antibiotic concentrations (μgL^{-1}) in surface waters and urban
 277 lakes in different countries and regions. AMO = Amoxicillin, CIP = Ciprofloxacin, TMP =
 278 trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and DOX = Doxycycline.

Location	Sample type	AMO	CIP	TMP	NOR	SMX	Ref
Nairobi,kenya	river water	n.r	0.509	2.65	nr	13.765	(Ngumba et al., 2016)
Nairobi,kenya	river water	n.r	0.168	3.346	n.r	11.25	(Bagnis et al., 2020)
Nairobi,kenya	river water	n.r	n.r	9.48	nr	23.35	(K'oreje et al., 2012b)
Hanoi, Vietnam ^a	surface water	<LOQ - 1.126	<LOQ - 0.115	0.002-0.07	n.a	0.11 - 3.5	(Tran et al., 2019)
Africa ^a	surface water	n.r	nd -0.51	0.024 -6.95		nd - 13.8	(Madikizela et al., 2017)
Global ^b	surface water	n.r	18.99	0.037	3.457	0.095	(aus der Beek et al., 2016)

(aus der
Beek et al.,
2016)

Europe ^b surface waters n.r 0.002 0.012 0.004 0.033

279 a = Concentration range, b = average concentration, n.r = Not reported

280 *4.2 risk of evolution of antimicrobial resistance*

281 Various studies have been conducted on environmental pollution by pharmaceuticals and personal
282 care products (Fatta-Kassinos et al., 2011). However, less attention has been given to the risk
283 associated with the development and propagation of antimicrobial resistant bacteria and genes in
284 the hydrological cycles as a result of residual antibiotics. Besides their effect on larger aquatic
285 organisms, their impact on pathogenic bacteria, especially the selection of resistant strains, is of
286 great concern.

287 Generally, most of the antibiotics were measured above their compound-specific PNEC values for
288 resistance selection. Increased prevalence of antibiotic resistant bacteria could be a result of
289 environmental bacterial communities undergoing resistance selection pressure due to continuous
290 contact with residual antibiotics (Michael et al., 2013; Wu et al., 2018). It has been predicted that
291 resistance resulting from bacterial exposure to subinhibitory concentrations of antibiotics is
292 irreversible, even in the absence of the antibiotic, since the mutants are more stable than the
293 bacteria selected at higher concentrations (Sandegren, 2014). Enrichment and selective advantage
294 of resistant bacteria has also been confirmed at subinhibitory concentrations (Gullberg et al., 2011;
295 Liu et al., 2011).

296 The use of untreated wastewater for agricultural purposes was observed during sampling. This
297 potentially creates an enormous biosecurity risk by exposing the environment and food chain to
298 residual APIs. Antimicrobial resistance can be transmitted to humans and animals through the food

299 chain, by consumption of untreated water, or indirectly through environmental emissions.
300 Mortality due to drug resistant bacterial infections, like tuberculosis, is on the rise in Kenya, with
301 approximately 169 000 deaths reported in 2017, 30% of which were attributed to multi-drug
302 resistant bacteria (WHO, 2017). Furthermore, 36.7% multidrug resistance among *Klebsiella spp*
303 strains has been reported on the central and western regions of Kenya (Taitt et al., 2017). Further
304 research into AMR at the studied sites is needed.

305 **5. Conclusions**

306 This study presents a risk assessment of the prevalence and resistance selection of six antibiotics
307 (AMO, NOR, CIP, DOX, TMP and SMX), in the wastewaters, surface waters and river sediments
308 of four Kenyan wastewater treatment plants. Levels ranging from <0.1 to 56.6 μgL^{-1} were found,
309 which are comparable to values reported in other parts of Kenya, and two to three orders of
310 magnitude higher than data reported in the global North.

311 Presence of APIs in the sediment phase were also reported in this study. The findings present a
312 broader picture of the situation in two previously unexplored, relatively smaller counties besides
313 Nairobi and Kisumu, which have been studied previously. Low connectivity to a centralized
314 wastewater treatment network (<10%) could be the biggest driver directing discharge of untreated
315 waste into the water bodies.

316 In most cases, the antibiotic levels reported in this study were higher than the PNEC values for
317 resistance selection for multiple genera of pathogenic bacteria. This implies a medium to high risk
318 of selection for antibiotic resistance within the respective environmental compartments, a major
319 threat to human health.

320 Data presented in this paper from previously unexplored areas can help to improve the knowledge
321 and risk assessment of the levels of active antibiotics in the aqueous and sediment phases in
322 Kenyan waters. Based on this data, we recommend raising general public awareness of the possible
323 dangers of directly discharging human waste into water bodies. Local authorities in the study areas
324 are encouraged to increase access to sustainable sanitation solutions in order to mitigate the direct
325 discharge of wastewater into water bodies, especially within informal settlements. This
326 information will help healthcare stakeholders and policymakers to understand the possible sources
327 and drivers of antibiotic resistance within natural environments. It will also be beneficial in the
328 process of formulating strategies to mitigate antimicrobial resistance.

329 **Declaration of interests**

330 The authors declare that they have no known competing financial interests or personal
331 relationships that could have appeared to influence the work reported in this paper.

332

333 **Acknowledgments**

334 This work was financially supported by the University of Jyväskylä doctoral program in the
335 Department Biological and Environmental Science, the Academy of Finland (grant #314939) and
336 by Jane and Aatos Erkko Foundation. We acknowledge the support of Martin Murigi, Machakos
337 water and sewerage company (MACHWASCO), Meru water and sewerage services (MEWASS)
338 and Nyeri water and sewerage company (NYEWASCO) during sample collection. Lastly we
339 sincerely thank the three anonymous reviewers whose comments helped improve earlier drafts of
340 this manuscript.

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527

528

529

SUPPLEMENTARY MATERIAL

530

Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan

531

wastewaters, surface waters and sediments

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547 **Table S1:** sample collection sites

	Site	Sample type	Sample Code	
Machakos	WWTP 1	Influent	MA 1	
		Effluent	MA 3	
	Mitheu River	Surface water grab (200m upstream)	MA 4	
		river sediment (200m upstream)	MA 5	
		Surface grab (2 km upstream)	MA 6	
		Surface water grab (200m downstream)	MA 7	
		Mwania river	Surface grab (2 km downstream)	MA 8
		River sediment (2 km downstream)	MA 9	
	Nyeri county	Gatei WWTP 2	Influent	NY 1
Effluent			NY 3	
Sagana river		Surface water grab (1km downstream)	NY 4	
		river sediment	NY 5	
Kangemi WWTP 3		Influent	NY 6	
		Effluent	NY 8	
Chania river		Surface grab (2m downstream)	NY 9	
		river sediment(2m downstream)	NY 10	
		Surface water grab (5m upstream)	NY 11	
		river sediment (5m upstream)	NY 12	
		Surface water grab (200m)	NY 13	
		river sediment	NY 14	
Meru County		WWTP 4	Influent	ME 1
			Effluent	ME 3
	Kanyuru River	river source swamp (2km upstream)	ME 5	
		river sediment (2km upstream)	ME 6	
		surface grab (500m downstream)	ME 7	
		river sediment	ME 8	
		Surface grab downstream 1km	ME 9	
		river sediment	ME 10	

548

549 **Table S2:** physicochemical properties of selected API's

compound	¹ molecular formula	¹ CAS No.	² water solubility mgL ⁻¹	² Excretion as parent compound (%)	³ log K _{ow}
Doxycycline (DOX)	C ₂₂ H ₂₄ N ₂ O ₈	564-25-0	630	70	
Amoxicillin (AMO)	C ₁₆ H ₁₉ N ₃ O ₅ S	26787-78-0	958	60-80	
Sulfamethoxazole(SMX)	C ₁₀ H ₁₁ N ₃ O ₃ S	723-46-6	610	15-25	0.89
			400	80-90	0.91
Trimethoprim (TMP)	C ₁₄ H ₁₈ N ₄ O ₃	738-70-5			
	C ₁₇ H ₁₈ FN ₃ O ₃		80	80	0.28
Ciprofloxacin (CIP)		85721-33-1			
Norfloxacin (NOR)	C ₁₆ H ₁₈ N ₃ O ₃ F	70458-96-7	13500	60	

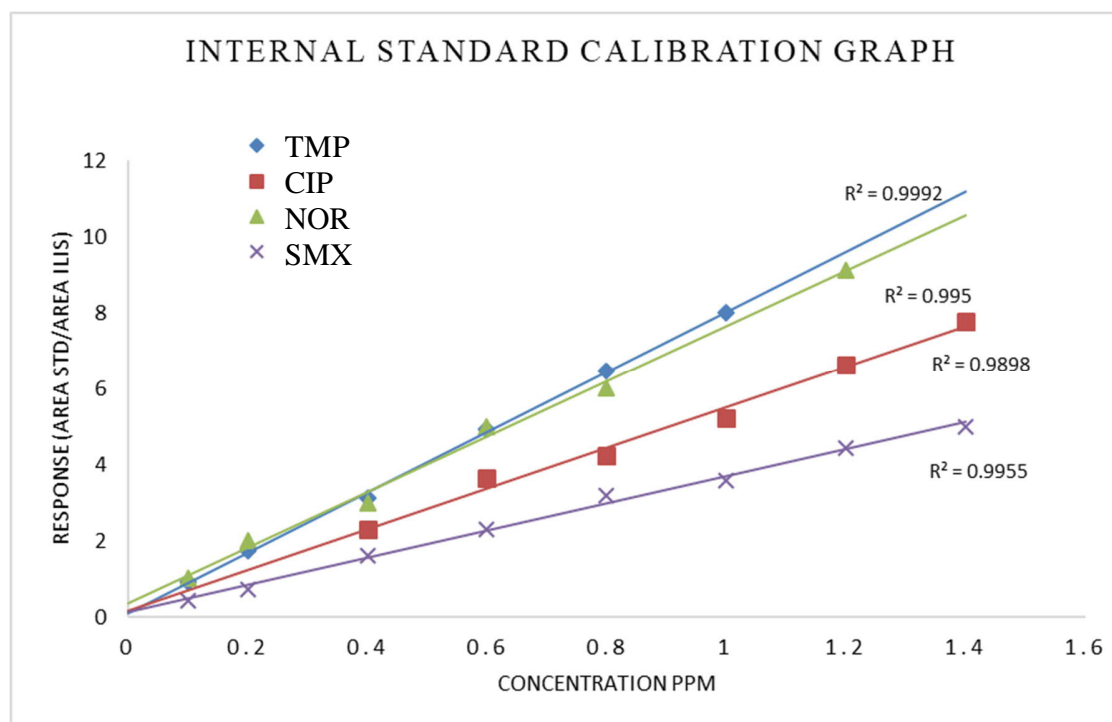
¹ Drugbank www.drugbank.ca ² Ngumba et al., 2016b ³ Madikizela et al., 2017

550 **Table S3:** Optimized LC-ESI-MS/MS instrument parameters for the analysis of the target
 551 compounds

Target compound	ILIS ^a	RT (Sd) ^b	Precursor ion [M+H] ⁺ (m/z)(CV) ^c	Quantifier ion (m/z) (CE) ^d	Qualifier ion (CE)
TET	n.a	2.63 (0.13)	445.0 (25)	154.0 (25)	410.0 (20)
AMO	n.a	1.77	365.9 (15)	113.9 (19)	348.9 (9)
CIP	[² H ₈]-CIP	2.24 (0.08)	332.1 (34)	288.0 (19)	314.1 (19)
TMP	[² H ₉]-TMP	2.25 (0.05)	291.1 (34)	123.0 (19)	230.0 (19)
NOR	[² H ₈]-NOR	2.15 (0.06)	320.3 (30)	276.0 (18)	302.0 (25)
SMX	[² H ₄]-SMX	4.83 (0.02)	254.0 (28)	156.0 (18)	108.0(17)
DOX	n.a	5.87 (0.01)	445.4 (30)	428.0 (25)	410.1 (25)

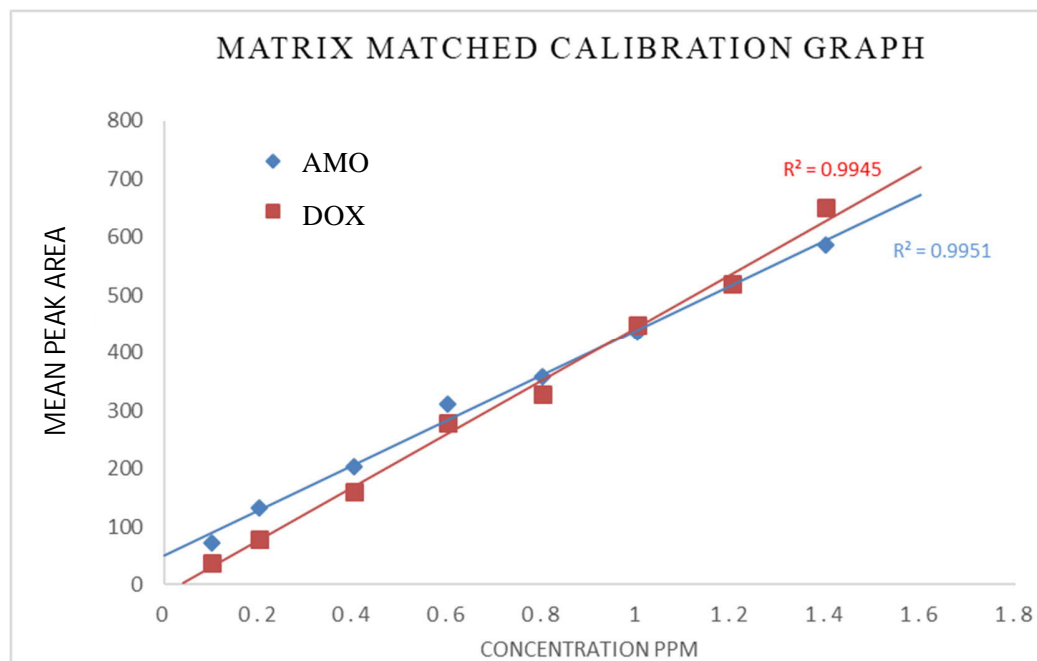
552 ^aILIS isotopically labelled internal standard. ^bRT retention time. ^cCV collision voltage ^dCE collision energy.
 553 n.a not available¹

554



555

556 **Figure S1:** Calibration graph for TMP, CIP, NOR and SMX constructed by plotting the ratio of
557 the Area of the standard divided by the area of the isotopically labelled internal standard against
558 the concentration.



559
560 **Figure S2:** Matrix matched calibration graph for AMO and DOX constructed by spiking surface
561 water at concentration levels between 0ppm (blank) and 1.4ppm

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