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Pius Kairigo

Occurrence and Fate of Antimicrobials and Antibiotic Resistance Genes in Urban Hydrological Cycles

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

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Diss.

Presence of active pharmaceutical ingredients (APIs) in the environment below therapeutic doses is accessory to driving resistance selection in micro-organisms. This study evaluated the measured environmental concentrations (MEC) of selected antimicrobials and antibiotic resistance genes in selected urban hydrological cycles in Kenya and Zambia. The MEC for selected APIs in aqueous, suspended particulate matter (SPM), and sediment samples ranged between < 0.1 to 956 μg l^{-1}; < 0.1 to 82267 μg kg^{-1}; < 0.1 to 4125 μg kg^{-1}, respectively. Emission of some APIs was through the SPM phase which shows that SPM is an important phase for investigation. Profiles of 33 clinically important antibiotic resistant genes (ARGs) and 2 integrons (*intl1_1* and *intl1_2*) were analyzed from samples collected in Lusaka Zambia. ARGs against sulfonamides, trimethoprim, tetracycline, quinolones, macrolides, rifamycin and *β*-lactams (including carbapenems), showed the highest average relative gene abundance of 10^{-3} to 10^{-1} genes per 16S rRNA gene. The *β*-lactamase gene bla_{TEM} had the highest relative abundance (10^{-2}, indicating 1 gene copy per 100 bacteria) in sediment samples fecal sludge and in Manchinchi WWTP influent water, whereas in water samples the relative abundance was one log lower (10^{-3}). Widespread detection of antibiotics and ARGs in surface water poses increased risk for downstream environments, as well as animals and humans who are exposed to contaminated surface water. Removal of model API from real urine by adsorption using wood and peat based powdered activated carbon (AC) showed overall removal > 93 %. The adsorption capacity of the AC in urine and pure water ranged between 0.75–16.6 mg g^{-1} and 10–200 mg g^{-1} respectively. This could be attributed to the urine matrix effects. Removal of APIs by AC offers possibility to further develop simple, robust, and sustainable treatment technologies for point-of-source mass reduction method.

Keywords: Antibiotic resistance genes; antimicrobials; risk control; wastewater.

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TIIVISTELMÄ

Kairigo, Pius
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Diss.

Lääkeaineiden esiintyminen ympäristössä voi aiheuttaa ympäristölle ja terveydelle riskejä. Varsinkin antimikrobiset lääkeaineet voivat aiheuttaa resistenssin syntymistä ympäristössä jo terapeuttista annosta pienempinä pitoisuuksina. Tässä tutkimuksessa mitattiin valittujen antibioottien sekä antiretroviraalisten lääkeaineiden pitoisuuksia (MEC) urbaanissa hydrologisessa kierrossa Keniassa ja Sambiassa. Tutkittujen lääkeaineiden pitoisuus jätevedessä vaihteli välillä <0,1–956 µg l^{-1}; jäteveden kiintoaineessa 0,1–82267 µg kg^{-1} ja sedimenttinäytteissä 4125 µg kg^{-1}. Jäteveden sisältämä kiintoaine on siis merkittävä reitti ympäristöön. Sambian Lusakasta kerätyistä ympäristönäytteistä analysoitiin 33 kliinisesti tärkeän antibioottiresistentin geenin ja kahden resistenssigeenien liikkumiseen liittyvän integronin (*intl1_1* ja *intl1_2*) profiilieja. Resistenssigeenejä, jotka antavat vastustuskyvyn sulfonamideja, trimetopriimiä, tetrasykliiniä, kinoloneja, makrolideja, rifamysiiniä ja β-laktaameja (mukaan lukien karbapeneemit) vastaan esiintyi näytteissä runsaasti. Niiden suhteellinen esiintyvyys vaihteli 10^{-3}–10^{-1} kopiota 16S rRNA -geeniä kohti. *β*-laktamaasigeeni, bla_{TEM}, oli suhteellisesti useimmin esiintyvä resistenssigeeni (10^{-2}, tarkoittaen 1 geenikopiota 100 bakteeria kohti) sakokaivolietteen käsittelyaltaan sedimentissä ja Manchinchin jäteveden puhdistamolle tulevassa jätevedessä, kun taas vesinäytteissä suhteellinen antibioottiresistenttien geenien runsaus oli kymmenesosa tuosta määrästä (10^{-3}). Antibioottien ja niille resistenttien geenien runsas esiintyminen ja päätyminen pintavesiin lisää riskiä alajuoksun ympäristöille sekä saastuneelle pintavedelle altistuville eläimille ja ihmisille. Normaaliin virtsaan lisätyt tutkittavat lääkeaineet poistuivat puusta ja turpeesta valmistetun jauhemaisen aktiivihiilikäsittelyn avulla tehokkaasti, kokonaispoistuma oli yli 93 %. Lääkeaineiden adsorptiokapasiteetti todellisessa virtsamatriisissa oli 0,75–16,6 mg g^{-1} verrattuna puhtaaseen veteen 10–200 mg g^{-1}. Tämä johtuu siitä, että virtsassa kilpailevan matriisin pitoisuus on useita kertaluokkia suurempi kuin lääkeaineilla. Silti lääkeaineiden poisto aktiivihiilen absorboitumalla tarjoaa mahdollisuuden kehittää yksinkertaisia ja edullisia käsittelymenetelmiä lääkeaineiden poistamiseen erilliskerätystä virtsasta.

Avainsanat: Antibioottiresistenssigeeni; antiimikrobiaaliset lääkeaineet; jätevesi; riskinhallinta.
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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their roman numerals I-IV. The responsibilities and contributions of authors are given in Table 1.

I 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, 137580.

II Kairigo P., Ngumba E., Sundberg L.-R., Gachanja A. & Tuhkanen T. 2020. Contamination of surface water and river sediments by antibiotic and antiretroviral drug cocktails in low and middle-income countries: Occurrence, risk and mitigation strategies. *Water* 12, 1376.

III Kairigo P., Laukkanen P., Ngumba. E, Nyirenda J., Kasoma N., Gachanja A., Tuhkanen T. & Sundberg L.-R. 2024. Presence of antibiotics and antibiotic resistant genes in urban hydrological cycles of Lusaka, Zambia using high-throughput quantitative PCR analysis. *Manuscript.*

IV Kairigo P., Ilomäki J., Ngumba E., Ouma J., Otieno A., Madivoli E., Gachanja A. & Tuhkanen T. 2024. Removal of antibiotics and antiretroviral drugs from hydrolyzed urine using activated carbon**.** *Submitted Manuscript*.

TABLE 1 The contribution of different authors to papers I–IV. PK=Pius Kairigo, TT=Tuula Tuhkanen, LS=Lotta-Riina Sundberg, AG=Anthony Gachanja, EN=Elijah Ngumba, JO=Josephine Ouma, AO=Austine Otieno, EM=Edwin Madivoli, JI=Johannes Ilomäki, JN=James Nyirenda, NK=Nicholas Kasoma, PL=Pinja Laukkanen, DOP=Daniel de Oliviera Patricio.

	I	II	III	IV
Conceptualization	PK,EN,LS,AG,TT	PK,EN,LS,AG,TT	PK,LS,TT,EN,AG	TT,PK,EN,AG
Experimental design	PK,EN,AG,TT	PK,EN,AG,TT	LS,TT,PK,DOP	TT,PK,JI,OA, EN,JO
Data collection	PK,EN,AG	PK,EN,AG	LS,PK,TT,JN,NK,PL,DOP	PK,EN,JO,JI
Data analysis	PK,EN	PK,EN	PK,LS,JO,EN,PL,DOP	PK,EN,JO,EM,JI
Writing- original draft	PK	PK	PK	PK
Writing- review and editing	PK,EN,LS,AG,TT	PK,EN,LS,AG, TT	PK,LS, TT, EN, JO, NK, JN, PL,DOP	PK,EN,JI,JO,AG EM,TT

ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired immunodeficiency syndrome
AMO	Amoxicillin
ABR	Antibiotic resistance
AMR	Antimicrobial resistance
AOPs	Advanced oxidation process(es)
APIs	Active pharmaceutical ingredient(s)
ARG	Antibiotic resistance gene
ART	Antiretroviral therapy
ARVDs	Antiretroviral drug(s)
BET	Brunauer-Emmett-Teller
CAS	Conventional activated sludge system
DOC	Dissolved organic carbon
HGT	Horizontal gene transfer
HICs	High income countries
HIV	Human immunodeficiency virus
LC	Liquid chromatography
LMICs	Low- and medium-income countries
LOD	Limit of detection
LOQ	Limit of quantitation
MDR	Multidrug resistance
MEC	Measured environmental concentrations
MGE	Mobile gene element
MQ	Milli-Q Ultrapure water
MS/MS	Tandem mass spectrometer
NOR	Norfloxacin
NVP	Nevirapine
PAC	Powdered activated carbon
PE	Population equivalent
PNEC	Predicted no effect concentration
q-PCR	Quantitative polymerase chain reaction
RD	Research and development
RQ	Risk quotient
RS	Resistance selection
SMX	Sulfamethoxazole
SPE	Solid phase extraction
SPM	Suspended particulate matter
SSU	Source separated urine
TMP	Trimethoprim
WSP	Waste stabilization ponds
WWTP	Wastewater treatment plant

1 INTRODUCTION

1.1 General introduction

Antimicrobials constitute a group of active pharmaceutical ingredients (APIs) that are an essential part of successful management of infections, thereby curing fatal and otherwise difficult diseases, increasing human and livestock lifespans, and raising quality of life (Kümmerer 2009a, Gelband *et al.* 2015, Larsson and Flach 2021). Globally, pharmaceutical production and consumption trends including antibiotics has increased, largely driven by factors such as: (i) increased disease burden; (ii) aging populations; (iii) ease in accessibility due to expiring patents; (iv) availability of antibiotics over the counter; and (v) rising incomes especially in developing countries. Between years 2000–2015 the rate of antibiotic consumption by humans increased by more than 65 % defined daily doses with low- and medium-income countries (LMICs) having the bulk of consumption (Klein *et al.* 2018, Patel *et al.* 2019). However, antimicrobials consumed by humans and animals are often poorly metabolised in the liver and thus excreted as parent compounds or active metabolites (Zhou *et al.* 2021a, Serrano *et al.* 2021, Imwene *et al.* 2022). These enter the environment through the centralized and decentralized sanitation systems, or to the receiving water bodies as a direct discharge of untreated excreta. APIs in the environment are accessory to environmental dimensions for development of antimicrobial resistance (AMR) (Larsson and Flach 2021, Kirchhelle 2023).

Previous studies have shown that conventional wastewater treatment plants (WWTPs) do not sufficiently remove APIs including antibiotics (average removal range between 10–90 %), mainly because of the slow microbial degradation of recalcitrant pharmaceuticals. Furthermore, the APIs are pseudo persistent resulting to ubiquitous detection in the urban hydrological cycle (Kümmerer 2009a, Visca *et al.* 2021). This implies that even in developed countries with well-maintained sewage networks, the WWTPs are point sources of antibiotics to the environment (Ngumba *et al.* 2016b, Wang and Wang 2016). The situation in LMICs is of concern, especially where there is non-existing sanitation systems or the existing sanitation treatment systems are non-functional (Michael *et al.* 2013, Nantaba *et al.* 2020, K'oreje *et al.* 2020). For instance, some of the studied WWTPs in this study were designed to release biosolids through the effluent. This is due to absence of desludging and sludge

recirculation mechanisms, leaving the opportunity for release of deconjugated, resuspended and sorbed APIs through the effluent and effluent suspended particulate matter (Jelic *et al.* 2015, Polesel *et al.* 2016).

The increased use of antimicrobials especially antibiotics, and eventual discharge into the environment create a selective pressure resulting in the emergence of antimicrobial resistance (Zhou *et al.* 2021b). For instance, antibiotic resistance is currently considered as one of the most urgent threats to modern healthcare. In recent times, the role of the environment in development of resistance is increasingly recognized (Prestinaci *et al.* 2015, Dadgostar 2019, Murray *et al.* 2022, 2024). Previous studies show that the development of antibiotic resistance is connected with human activities including overuse and misuse of antibiotics in human and veterinary medicine (Kümmerer 2008, 2009b, Vikesland *et al.* 2017). These issues are further amplified in LMICs where socioeconomic factors dictate the handling of antibiotics (Collignon *et al.* 2018). For instance, it is a common practice in low- and medium-income countries (LMICs) in this study for patients to self-diagnose and self-medicate due to unavailability and inaccessibility to healthcare, easy access to over-the-counter drugs and poor drug regulatory measures. This could potentially lead to wrong diagnosis, under-medication, or over-consumption of antibiotic drugs (Oyediran *et al.* 2019). Furthermore, in LMICs the regimen for antibiotic and antiretroviral drugs (ARVDs) used in the fight against human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) and other opportunistic infections contribute to increased consumption of antibiotics and ARVDs per capita, particularly in Sub-Saharan Africa. Besides the antibiotic resistance development, concerns over antiviral resistance evolution have increased due to the massive environmental contamination especially in areas with high HIV/AIDS patients (Ncube *et al.* 2018, Nannou *et al.* 2020).

The prevalence of antimicrobial resistance and its wider implications present a growing healthcare crisis of post-antibiotic era declared by the WHO, where common infectious diseases will be among the leading causes of global mortality by 2050 (Prestinaci *et al.* 2015, Gautam 2022). The situation is worsened by the possible spread of antimicrobial resistance, resulting to co-morbidity and mortality among populations. For instance, colistin resistance gene that emerged from pig farms in China, has since spread and detection made elsewhere across the globe within few years (Reardon 2017, Zhang *et al.* 2021). It is already estimated that by 2050, over 10 million people could die annually due to AMR related causes (Gautam 2022, Murray *et al.* 2022). The environment is an important factor in the transmission of AMR (Larsson *et al.* 2018). Ability to control environmental contamination by excreta is paramount in implementing multibarrier approach to environmental dimensions of antibiotic resistance development and transmission. This is because, excreta, especially urine is among the point sources of residual APIs (Bischel *et al.* 2015, Viskari *et al.* 2018, Imwene *et al.* 2022). Countries with subservient wastewater treatment

infrastructure may contribute the bulk of environmental resistance transmission (Larsson and Flach 2021).

The net drivers and pathways of antibiotic resistance development in the environment are yet to be clearly understood due to the highly complex systems driving the antibiotic resistance. However, consensus exists that environmentally representative micro-contaminant concentrations of antibiotics facilitate antibiotic resistance gene (ARG) spread via mobile genetic elements, such as plasmids (Kim *et al.* 2014, Singer *et al.* 2016). Environmental microbiome has a massive diversity (compared to human and animal hosts treated with antibiotics) that can provide a wider resistance gene pool for acquisition by pathogenic microorganisms, which can impede activity of antibiotics (Bengtsson-Palme and Larsson 2015, Larsson and Flach 2021).

Based on the negative effects associated with the presence of antimicrobials in the environment, their control and removal is critical. Mitigating entry of APIs into the urban hydrological cycles from known sources is critical instead of the reliance on end of pipe technologies which are inefficient in API removal (Serrano *et al.* 2021). Active discussions are ongoing concerning the allocation of responsibility between key actors for managing antibiotics in the environment and the control of antimicrobial resistance (AMR) formation, with proposals pushing for 'polluter pays' model (UNEP 2022, Malmqvist *et al.* 2023). For instance, the European commission included 'prevention and control' in the AMR action plan as one of the key areas of focus (EC 2017). While existing WWTPs are not typically equipped to effectively capture and remove pharmaceutical residues, some European countries such as Switzerland, Germany, Finland, Netherlands, and Sweden have at least calculated the extra cost for post-treatment options or have already started adapting advanced post-treatment techniques such as combination of ozonation and adsorption to control the flow of APIs (Michael *et al.* 2013, Pistocchi *et al.* 2022b).

1.2 Antibiotic resistance in the environment

1.2.1 Environmental dimensions of antimicrobial resistance

Emergence and spread of antibiotic resistance genes in the environment is a threat to public health. This is due to potential impact to human microbiome resulting from selection for antibiotic resistant bacteria (Brinkac *et al.* 2017, Lu *et al.* 2023). Although intrinsic antibiotic resistance within microbial communities exists, the acquired resistance arising via mutations in chromosomal genes and, more importantly, by acquisition of external genetic determinants is selected under the influence of antibiotics (Munita and Arias 2016, Larsson and Flach 2021). Mobilization of this acquired resistance to new hosts and their expression under different contexts is a

cause for concern and the main cause of modern problems with antibiotic resistance (Peterson and Kaur 2018, Larsson and Flach 2021). Presence of antibiotic resistant bacteria and antibiotic resistance genes in the environment have been previously reported in literature (Hunter *et al.* 2008, Sobsey *et al.* 2014, Prestinaci *et al.* 2015, Sabri *et al.* 2018, Hendriksen *et al.* 2019, Nadimpalli *et al.* 2020, Zhuang *et al.* 2021, Xu *et al.* 2024).

Selection of high-level resistance in successive bacterial generations can result from overuse of antimicrobials as well as usage of sub-lethal concentrations (Reygaert 2018). Therefore, presence of residual APIs in the environment has potential to mediate antimicrobial resistance in the environment whereby microbial organisms are exposed to antibiotics in sub-lethal doses (Kümmerer 2003). Under these conditions, the resistant bacteria have selective advantage, leading to their enrichment in the population (Gullberg *et al.* 2011, Li *et al.* 2016, Emara *et al.* 2023). Sub-lethal doses give competitive advantage to growth of resistant strains that will become harder to treat (Lindberg *et al.* 2005, Liu *et al.* 2011, Andersson and Hughes 2014, Li *et al.* 2016).

Widespread environmental releases of pollutants such as the APIs, ARBs, and ARGs emanate from discharge of untreated human and animal excreta into the environment (Kotwani *et al.* 2021). Furthermore, untreated wastewater from manufacturing of antimicrobial compounds contributes APIs in the environment (Bengtsson-Palme *et al.* 2019). The release of AMR pollutants to the environment occurs when containment barriers are missing or not working effectively.

Poor sanitation is direct contributor to the AMR contaminants in the environment because properly functioning, adequate and efficient sanitation systems are critical control barriers to direct discharge of untreated wastewater (Graham *et al.* 2019, Samreen *et al.* 2021, Sambaza and Naicker 2023). Furthermore, incompletely treated effluent wastewater from pharmaceutical manufacturing and hospitals may contribute largely because of elevated concentrations of diverse antimicrobial compounds including last resort compounds, pathogens, and resistant microbes (Kotwani *et al.* 2021, Kusi *et al.* 2022). Agricultural activities especially in food crop and animal production also contributes AMR contaminants in the environment due to the discharge of large amounts of fungicides, herbicides, antimicrobials and resistant microbes through inappropriate disposal or untreated discharge of waste streams (UNEP 2022). Furthermore, there are rising concerns over the use of biosolids in agricultural activities (Sharma *et al.* 2017, Pozzebon and Seifert 2023).

1.2.2 Bacterial genomic flexibility and horizontal gene transfer

Genomic flexibility denotes the exchange of genetic material among organisms, either conspecific or heterospecific, impacting the host's genetic, physiological, and ecological performance (Emamalipour *et al.* 2020). Bacteria, characterized by genomic flexibility, have amassed substantial diversity, enabling adept adaptation to diverse ecological niches (Miller *et al.* 2009, Jiao *et al.* 2024). This adaptability predominantly stems from mobile genetic elements (MGEs), comprising transferable DNA units within or between genomes, thereby facilitating horizontal gene transfer (HGT). This involves the acquisition of novel traits by the recipient lineage, culminating in the

transmission of heritable genetic information not explicable by conventional vertical parent-offspring transmission (Hall *et al.* 2017, Brito 2021). The main mechanisms of HGT include transduction, conjugation, and transformation which collectively encompass DNA transfer via bacteriophage, in contact cell-to-cell transfer, and via free DNA uptake from the environment, respectively (Blakely 2015). Recent revelations indicate that bacteria can also convey DNA fragments through membrane-bound vesicles and nanotubes (Emamalipour *et al.* 2020). Despite the HGT mechanism, a comprehensive understanding of the specifics within natural microbial communities remains constrained due to challenges inherent in situ examination of the mobile gene pool. However, strides in metagenomic sequencing provide avenues for probing these intricate questions.

MGEs exert profound effects on host cells, endowing them with traits such as antibiotic resistance, carbohydrate digestion, mercury resistance, virulence, and catabolism pertinent to bioremediation (Brito 2021). HGT additionally contributes to the augmentation of bacterial genome size through the incorporation of sequences, for instance as genomic islands (Juhas *et al.* 2009, Rodriguez-Valera *et al.* 2016). Moreover, HGT assumes a pivotal role in steering bacterial genome evolution, structural dynamics, and environmental adaptation, encompassing the colonization of novel environments, the exploitation of diverse carbon sources, and fortification against toxins (Hall *et al.* 2017). Bacterial features conducive to HGT-mediated evolution include heightened reproductive activity, ensuring the transference of mutations and acquired genetic material across successive generations. The absence of membrane-bound nuclei in bacteria amplifies the accessibility of their genomes to incoming DNA, thereby facilitating the acquisition and integration of novel genes (Brito 2021).

1.2.3 Analysis of ARGs in the environment

In recent times, there is growing interest in characterization of environmental resistome profiles because of the increasing realization that the environment is an important vector in development and dissemination of AMR. Currently there are a growing number of methods for characterization of phenotypic resistance (identification of resistant bacteria) and genotypic resistance (characterization of resistant genes). Phenotypic methods are mainly culture-based and include methods such as disk diffusion, breakpoint agar test, and broth microdilution method. These methods are relatively cheap, easy to perform, sensitive and have high reproducibility of results. However, they are limited because of the extended periods of time needed by some microorganisms to multiply and the inability to detect presence of valid but non-cultivable microorganisms (Anjum 2015). Genotypic characterization of ARGs mainly involves molecular methods whose principle is based on amplification of target genes. These include PCR based methods (such as conventional PCR, real-time PCR (qPCR), digital PCR among others) and DNA microarray. Resistance genes can be also found using whole-genome sequencing or metagenomics, and the activity of the genes can be detected using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF-MS) (Anjum 2015, Galhano *et al.* 2021). PCR-based molecular methods have advantages which include the high sample

throughput and fast execution time. Furthermore, using metagenomics, it is possible to identify all resistomes in a sample. Some limitations of molecular methods include expensive special equipment and a trained operator, and the need for bioinformatic expertise for data interpretation and analysis (Galhano *et al.* 2021). The choice of method to use is influenced by of information sought, the threshold for detection whether it is targeted gene analysis or whole genome sequencing among other factors such as cost and availability of technology. Therefore, qPCR is a common method in ARG surveys, because of the fast reaction times and ability to quantify relative and absolute abundance of the targeted resistance genes.

1.3 Sources and fate of antimicrobials in the environment

Extensive studies on presence of pharmaceutical residues in the environment exist in literature and measured environmental concentrations have been reported globally (Kümmerer 2009b, Hanna *et al.* 2018, Wilkinson *et al.* 2022). Specific studies to survey MECs in wastewaters and recipient waters in Finland and Sub-Saharan Africa have also been made (Vieno *et al.* 2007, Ngumba *et al.* 2016b). The main sources of APIs in the environment include effluent from incompletely treated effluent from pharmaceutical industries, hospitals, agriculture, and domestic households. Domestic households (Figure 1) are considered point sources because of disposal of unused pharmaceuticals into the wastewater streams (Kasprzyk-Hordern *et al.* 2021, Rogowska and Zimmermann 2022). Furthermore, pharmaceuticals consumed by humans and animals do not metabolize completely in the body and therefore are excreted in urine or fecal matter in their original form or as pharmacologically active metabolites or transformational products (Jjemba 2006, Fan-Havard *et al.* 2013). The percentage of the API excreted in its original form differs based on the category of the compound and may be as high as 90 % (trimethoprim in urine) (Kümmerer 2009a, b, Tran *et al.* 2016, Li *et al.* 2021). The residual APIs and their active metabolites enter the hydrological cycles either by direct discharge into the environment or through incompletely treated wastewater effluents (Kümmerer 2009b, Petrovic *et al.* 2009, Matongo *et al.* 2015a, Zhang *et al.* 2015, Ngumba *et al.* 2016b, Tran *et al.* 2019). Once released into the treatment systems, APIs undergo biotic and abiotic abatement processes (Boxall 2004, Kaeseberg *et al.* 2018, Costa *et al.* 2019). However, removal of micropollutants of pharmaceutical nature, especially antibiotics, is incomplete in the current conventional treatment systems (Fatta-Kassinos *et al.* 2011, Wang and Wang 2016, Imwene *et al.* 2022). Furthermore, the removal of APIs can be due to adsorption to biosolids. However, in treatments systems lacking biosolid collection and treatment systems, desorption of API contaminants during resuspension, and deconjugation of conjugated metabolites back to parent compounds, can occur under favorable conditions (Radke *et al.* 2009, Bagnis *et al.* 2020). This influences the emission loads of APIs into receiving surface waters which contributes the ubiquitous detection of APIs in the surface waters.

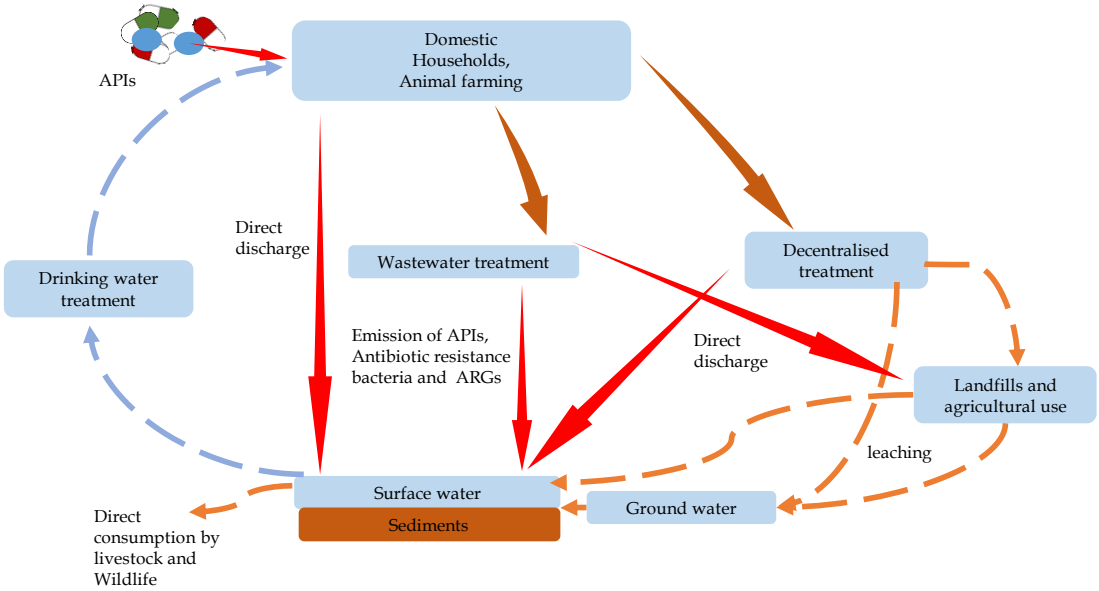

FIGURE 1 Pathways of APIs in the environment with illustrating the risk of emission of antimicrobial residues, antibiotic resistant bacteria, and genes into the water sources.

Understanding the physico-chemical properties (see Chapter 3 Table 4) of pharmaceutical compounds is crucial because they influence metabolism, distribution, and rate of environmental degradation. The important parameters to consider include octanol–water partition coefficient (log K_{ow}) which describes the partition of an analyte between the two phases, water solubility (S), Henry's law constant (H_c) acid dissociation constant (pK_a) which describes the degree of ionization, and biosolids/water distribution coefficient (K_{bio}) which describes adsorption to organic matter (Kümmerer 2008, Khasawneh and Palaniandy 2021, Ohoro *et al.* 2022). At a particular *pH*, *pKa* values are important for describing the availability to biological organisms and chemical and physical reactivity which describes its environmental fate (Kümmerer 2008). Several other factors can influence the behaviour of API residues in the environment including solution *pH*, water quality, temperature, microbial communities, tidal factors, and hydraulic retention time among others (Joss *et al.* 2005, Zhao *et al.* 2015, Saleh *et al.* 2020).

1.4 Urine source separation and treatment

Adequate water supply and wastewater treatment are essential to reach several interconnected sustainable development goals (SDGs). Concerted efforts are focused on contributing provision of safe drinking water, adequate sanitation for all, as well as protection of water resources against pollution (SDG 6). Furthermore, urine source separation and effective treatment addresses SDG 14.1, on reducing nutrient emissions to the aquatic environment. It is increasingly evident that conventional water-based centralized sanitation practices are inadequate to achievement of

environmental health targets globally (Larsen *et al.* 2021a, Obaideen *et al.* 2022). Adoption of water-based centralized sanitation system in less developed countries is slow, expensive, and insufficient to cover all the population (Larsen *et al.* 2007, Onu *et al.* 2023). In addition, the ageing infrastructure of centralized sanitation in developed countries is inadequate to fulfill the emerging challenges of controlling chemicals of emerging concern (Köpping *et al.* 2020). Approximately 35 % of the global population is covered by centralized sanitation systems (Adhikari and Halden 2022). Therefore, innovative technologies that control the APIs point of entry to the urban hydrological cycle should be promoted. Decentralized dry sanitation with urine diversion, treatment and value addition could aid towards faster achievement of the SDGs related to health, nutrition, and resource recovery (Öberg *et al.* 2020).

Urine contributes approximately 1 % by volume of the total wastewater (Barbosa *et al.* 2019, Zhou *et al.* 2021a), but consists approximately 70 % of APIs and the metabolites of ingested pharmaceuticals. Furthermore, urine contains approximately 80–90 %, 50–65 %, and 50–85 % of the N, P, and K respectively (Heinonen-Tanski *et al.* 2010, Viskari *et al.* 2018). Presence of nutrients and micropollutants in the wastewater effluent poses a threat to the ecosystem in all ecological realms, as the receiving surface water bears the burden of damage. Discharge of essential nutrients to surface water is responsible for the rise in eutrophication in surface water bodies across the globe, a serious problem transcending planetary boundaries (Steffen *et al.* 2015). Low- and middle-income countries (LMICs) are particularly vulnerable due to inadequate wastewater collection, conveyance and treatment systems leading to direct discharge of domestic waste into the environment (Öberg *et al.* 2020).

Efficient handling of urine source separation within a system, where treated separated urine is purged of remaining pharmaceutical residues, has the potential to act as a formidable critical barrier against environmental contamination arising from human activities. This approach ensures the potential to recirculate vital plant nutrients, especially nitrogen and phosphorus. Separation of urine at source and repurposing it for fertilizer production, a self-sustaining nutrient cycle is fostered, which benefits the sanitation value chain and agriculture (Simha and Ganesapillai 2017, Demissie *et al.* 2023). This strategy not only supports responsible nutrient management but also provides a means to regulate the environmental influx of antimicrobial residues.

Studies show that human excreta can be used as fertilizer to grow crops and minimize loss of essential plant nutrients (Heinonen-Tanski and Van Wijk-Sijbesma 2005, Tran-Thi *et al.* 2017, Viskari *et al.* 2018, Kelova *et al.* 2021). However, uptake of residual pharmaceuticals by plants especially food crops is a cause for concern (Pan and Chu 2017, Akenga *et al.* 2021, Häfner *et al.* 2023). Based on literature values, API concentrations in SSU are usually more than 2 orders of magnitude higher than those in municipal wastewater treatment plants (Ngumba *et al.* 2020). This implies that direct use of sanitized SSU bears some risk of fertilizing the soil with pharmaceutical residues. This presents a need to develop efficient treatment methods for removal of pharmaceutical residues from urine to produce a safe fertilizer (Jermakka *et al.* 2021, Demissie *et al.* 2023). Source control measures should be taken first, because such

measures are usually far more effective when the volumes are smaller, and concentrations are higher (EurEau 2019). Furthermore, this would also decrease the pressure to enhance the downstream mitigation measures.

Presently, several methods of pharmaceutical removal, mostly from synthetic urine at laboratory scale have been evaluated. The common approach to urine treatment includes hygienization, reducing volume, stabilization, nutrient recovery, nutrient removal and addressing micropollutants (Larsen *et al.* 2021b). Adsorption of pharmaceutical residues in urine using biochars is an alternative cheap, effective and low-cost method of treatment (Chauhan *et al.* 2023). Adsorption of APIs using biochar was studied with >90 % removal of sulfonamide antibiotics and human metabolite by biochar and biochar/H_2O_2 in synthetic urine (Solanki and Boyer 2017, Sun *et al.* 2018). However, the residual API concentration after the biochar treatment were above the proposed PNEC values for resistance selection (Bengtsson-Palme and Larsson 2016) and therefore potentially unsafe for release to the environment. World Health Organization (WHO) proposed a urine storage time of at least six months to achieve pathogen inactivation (WHO 2006). However, the recommended six months storage can only partially remove the pharmaceuticals (Jaatinen *et al.* 2016, Duygan *et al.* 2021). Other methods for treatment and resource recovery include struvite precipitation for phosphorus recovery, freeze-thaw, reverse osmosis, electrodialysis, microfiltration, ozonation, ammonia stripping, anaerobic ammonium oxidation, electrolysis, distillation, ion exchange and nitrification (Maurer *et al.* 2006, Larsen *et al.* 2021b, Liu *et al.* 2022, Imwene *et al.* 2022). These methods are applicable separately or in combination (Table 2). For instance, a recent study showed removal of 75 micropollutants (mostly APIs) by photodegradation using ultraviolet light with efficiencies of >99 % in water and 55 % (±36) in fresh urine samples (Demissie *et al.* 2023). Use of combinations of treatment technologies has been shown to achieve better API removal efficiency (Sun *et al.* 2018, Köpping *et al.* 2020, Duygan *et al.* 2021).

Urine diverting systems (UDS), or ecological sanitation systems have been studied, piloted, and demonstrated for the control of pathogens, organic matter, and eutrophication. The possibility to provide sanitation, conserve water, and recover fertilizer was considered as the main drivers of UDS. Unfortunately, the technology has not been adapted into the wider use and data is only limited to pilot studies (Ishii and Boyer 2015, Larsen *et al.* 2021b). Furthermore, large scale commercialization has been hampered by socio-technological challenges in the operation and maintenance compared to conventional water-based sanitation (Larsen *et al.* 2021a). The possibility to control the flow of problematic pharmaceuticals and AMR bacterial load and prevention of further evolution of resistance genes can incentivize development of the UDS technology and operation, collection, treatment, and safe disposal of sanitation byproducts (Inyinbor *et al.* 2018). Additional relevant driver includes employment creation especially in the LMICs, through service provision and maintenance of the technologies.

TABLE 2 Application of various urine treatment technologies either singly or in combination for the removal of antimicrobials in human urine.

Target compound	Treatment method	Removal efficiency (%)	Comments	Reference
Degradation of 75 organic micropollutants in fresh urine and water	UV-(185,254nm)	99 % (±4) in water 55 % (±36) in urine	- No reduction of nitrogen content during treatment - UV treatment can reduce the load of organic micropollutants to urine recycling systems. - Dissolved organic matter in urine affected UV degradation in urine - 10 fold more energy needed in urine matrix than water	Demissie *et al.* (2023)
Nutrient recovery, removal of 11 spiked APIs (candesartan, carbamazepine, clarithromycin, diclofenac, emtricitabine, hydrochlorothiazide, irbesartan, metoprolol, N_4-acetylsulfamethoxazole, sulfamethoxazole and trimethoprim) in urine	Storage (74 days), nitrification and distillation, column adsorption with *GAC	>90 % (APIs)	- Pilot study, synthetic urine - The nutrient compounds (ammonium, nitrate, phosphate, potassium, and sulfate) were not removed significantly compared to APIs - Amount of GAC needed to remove APIs from **SSU reduced by close to two-fold, if urine were treated on site instead of being discharged and treated in a centralized WWTP. - Complete API removal was achieved for up to 660 bed volumes at an empty bed contact time (EBCT) of 70 min	Köpping *et al.* (2020)
Removal of spiked APIs (acetylsalicylic acid, paracetamol, ibuprofen, naproxen, citalopram, carbamazepine, and diclofenac)	Adsorption using Biochars (Activated coconut carbon, bamboo, and southern yellow pine biochars)	>90 %	- Synthetic urine used. - High concentration of nitrogen and phosphorus remains after biochar treatment	Solanki and Boyer (2017)
Removal Of sulfonamide APIs (sulfamethoxazole, sulfadiazine, sulfamethazine, sulfadimethoxine, metabolite, N4-acetyl-sulfamethoxazole)	-biochar/ H_2O_2		- Synthetic urine was used - H_2O_2 was added to create biochar/ H_2O_2 catalytic system - The metabolite, behaved differently from the parent indicating the importance of studying metabolites.	Sun *et al.* (2018)

Nutrient recovery (Antibiotics, antivirals, beta blockers analgesics, and diuretics)	Struvite precipitation, Nitrification distillation, electrolysis	Near complete nutrients recovery final product	- Real urine samples - Nitrification followed by distillation eliminates pathogens. - There is high nutrient loss in the effluent if struvite precipitation is not combined with other methods. - No API removal	Udert *et al.* (2015)
Antibiotics (30 common sulfonamides, tetracyclines and quinolones) Tetracycline resistant genes (*tet* A, *tet* Q, and *tet* M ARGs in urine	Storage at 27.1 °C ± 0.8 °C for 30 days	Antibiotics 27 % to 97 %	- Fresh and hydrolized urine used - Batch experiment - ARGs no change	Zhou *et al.* (2021a)
Recovery of total ammonia nitrogen (TAN)	Electrochemical ammonia stripping	58% (recovery)	- Synthetic urine used - Recovery was limited by TAN transport from the feed to concentrate compartment.	Kuntke *et al.* (2018)
Antibiotics, human metabolite, and antiretrovirals (atazanavir, atenolol, clarithromycin, darunavir, diclofenac, emtricitabine, hydrochlorothiazide, N_4-acetysulfamethoxazole, ritonavir, sulfamethoxazole, trimethoprim)	Storage, nitrification, aerobic biological treatment, adsorption using PAC	Storage 6−50 % MBBR 30−> 90 % PAC 20−> 90 %	- Real urine used - 60-day storage was insufficient for removal of APIs - Post-treatment using PAC was necessary to significantly remove APIs - No significant loss of nutrients after PAC adsorption.	Duygan *et al.* (2021)

*GAC = Granulated activated carbon, SSU= source separated urine

1.5 Technologies for removal of APIs, ABR and ARGs from wastewater

Conventional end-of-pipe water-based treatment systems are not efficient in the removal of micropollutants of pharmaceutical nature. Furthermore, these systems have an added challenge to counter the emergence of antimicrobial resistance bacteria and genes in the hydrological cycles. Instead of abatement of emerging environmental micropollutants, conventional wastewater treatment systems were found to be a gateway of residual APIs and resistance genetic material into the environment via effluent emissions and biosolids (Munir *et al.* 2011, Gupta *et al.* 2021, Rumky *et al.* 2022).

Wastewater treatment processes across the globe differ. This is because of varying composition, infrastructure, legislation and level adherence and implementation to universal wastewater treatment practices (Obaideen *et al.* 2022). Furthermore, climatic conditions, wastewater characteristics, type, and extent of pollutants especially emerging micropollutants such as pharmaceutical residues, vary greatly across geographical regions.

The common available treatment technologies are broadly classified as conventional, non-conventional and advanced post-treatment technologies. Conventional wastewater treatment methods mostly involve the centralized treatment plants that comprise a combination of physico-chemical and biological processes. Primary, secondary, or tertiary treatment stages may be applied to achieve the desired water quality features (Maddela *et al.* 2021). The most common conventional treatment systems include conventional activated sludge systems (CAS) with disposal of formed biosolids, trickling filters, rotating biological contactors and membrane bioreactors (MBR) (Gupta *et al.* 2021, Werkneh and Islam 2023). Non-conventional treatment includes systems such as waste stabilization ponds (WSPs), and constructed wetlands. They employ biological processes in treatment and are less complicated and costly in terms of operation and maintenance but require more land compared to conventional systems (Fahad *et al.* 2019).

The post-treatment technologies are tertiary steps that can be incorporated into either the conventional or non-conventional treatment processes to further improve the quality of the effluent. Common post-treatment methods include ozonation, adsorption, filtration, and UV photolysis (Kovalova *et al.* 2013, Rizzo *et al.* 2019, Ngumba 2020, Cheng *et al.* 2021, Werkneh and Islam 2023). Ozonation coupled with activated carbon adsorption (O_3 and AC) is among the best available technologies (BAT) for efficient elimination of micropollutants from wastewater (Rizzo *et al.* 2019, Cantoni *et al.* 2024). In Europe, O_3 and AC is the most adopted wastewater advanced post-treatment step (Pistocchi *et al.* 2022a). On the contrary in LMICs, conventional systems cover a small percentage of the population and mostly lack advanced post-treatment steps (Obaideen *et al.* 2022). Furthermore, treatment systems in LMICs lack

biosolid handling mechanisms such as removal or recovery from effluent. Most of the population in the study areas are served by decentralized systems which are inefficient for micropollutant removal or there is absence of wastewater collection and treatment systems all together, leaving an opportunity window for direct discharge of untreated excreta directly to the environment.

In terms of AMR, significant differences were reported between WWTPs emission of genetic material (ARGs and ARB) in biosolids and aqueous phases (Munir *et al.* 2011, Yang *et al.* 2014). This understanding can help reshape the focus of new technologies purposed for the elimination of ARGs. For instance, special attention should be focused on the biosolids, excess activated sludge and suspended matter not removed from less sophisticated treatment plants. There are numerous studies on elimination and removal efficiency of APIs and ARGs in water and wastewater (Table 3). The most studied methods are based on adsorption of APIs with low-cost adsorbents. Besides the adsorption, there are several other technologies utilizing biological, physical, and chemical treatments.

TABLE 3 Common wastewater treatment methods for removal of APIs, ARBs and ARGs

Target compounds ARG	Treatment method	Experimental conditions	Removal	Comments	Reference
7 antibiotics (erythromycin-H_2O, lincomycin, monensin, ofloxacin, sulfamerazine, sulfamethazine and novobiocin) 18 ARGs	- Constructed wetlands (CWS) - Substrates (oyster shell, zeolite, medical stone and ceramic)	- Horizontal subsurface-flow systems planted with *Cyperus alternifolius* L. - Used 3 hydraulic loading rates (HLR: 10 cm, 20 cm and 30 cm/day). - Antibiotic analysis- rapid-resolution liquid chromatography-tandem mass spectrometry (RRLC-MS/MS) - ARGs analysis- qPCR	Antibiotics 16 to 98 % ARGs 50 to 85 %	- Highest removal found in CWs with the lowest HLR under the same substrate condition - Removal for the total ARGs decreased with increasing HLR - CWs with zeolite and HLR 20 cm/day had best chemical removal. - Removal was by sorption and biodegradation	Chen *et al.* (2016a)
Antibiotics (erythromycin-H_2O, monensin, clarithromycin, leucomycin , sulfamethoxazole, trimethoprim, sulfamethazine, and sulfapyridine) ARGs (*sul*1, *sul*2, *sul*3, *tet*G, *tet*M, *tet*O, *tet*X, *erm*B, *erm*C, *cml*A, *flo*R, *int*1 and *int*2).	- Constructed wetlands (CWS)	- CWs with three flow types (surface flow, horizontal subsurface flowand vertical subsurface flow) - Two plant species (*Thalia dealbata* Fraser and Iris tectorum *Maxim*)	Antibiotics 75 to 98 % ARGs 64 to 84 %	- Removal influenced by biodegradation, adsorption on substrates and uptake by plants	Chen *et al.* (2016b)
ARGs (ermB, *tetA, tetC, tetQ, tetW, sul1 and intl1.)*	- Ultrasonication (WWTP sludge)	- 10 min of ultrasonication 700w then freeze-drying - Ultrasound frequency 20 to100 kHz - ARG analysis - droplet digital PCR	*tetA*, *tetC* and *tetQ* ≈ 10 *tetW, intI1 and ermB* +27	- ARGs not effectively removed by pre-treatment. - Long retention times (27 days) favored removal of ARGs in effluent - Higher relative abundance of ARGs found in the treated sludge	Rumky *et al.* (2022)
ARBs and ARGs	- Membrane Biological Reactor (MBR)		2.57 to 7.06 logs reduction		Munir *et al.* (2011)
ARBs and ARGs	- Conventional utilities (Activated Sludge, Oxidative Ditch and Rotatory Biological Contactors-RBCs)		2.37 to 4.56 logs reduction		Munir *et al.* (2011)

2 OBJECTIVES OF THE STUDY

This thesis aimed at investigating occurrence and fate of selected antimicrobials and antibiotic resistance genes in samples from urban hydrological cycles in Kenya and Zambia.

The specific objectives included:

i. Measure the occurrence and phase partitioning, for selected antibiotics in hydrological cycles of Kenya and Zambia (I, II).
ii. Assess the aquatic risk quotient for evolution of antibiotic resistance based on measured environmental concentrations for the selected antibiotics (I, II, III).
iii. Analyze selected antibiotic resistant gene profiles of treated and untreated wastewater, surface water, ground water, sediments and sewage sludge (III).
iv. Assess the feasibility for removal selected antibiotics and antiretroviral drugs from source separated urine using powdered activated carbon as a point-of source treatment technology (IV).

3 MATERIALS AND METHODS

3.1 Chemicals and standards

In this study, common classes of first line antimicrobials were selected (Table 4). These included antibiotics used for common ailments and antiretroviral therapy (ART) in the selected areas of study.

The target compounds included ten antibiotics, doxycycline (DOX), amoxicillin (AMO), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP) norfloxacin (NOR), ofloxacin (OFL), tetracycline (TET), erythromycin (ERY), and rifampicin (RIF). Three antiretroviral drugs (ARVDs), nevirapine (NVP), zidovudine (ZDV) and lamivudine (3TC) were included. The pharmaceutical standards were a kind donation from Universal corporation, a pharmaceutical manufacturer in Kenya. Isotopically labelled internal standards were purchased from Alsachim, France.

3.2 Study area and sample collection

Samples were collected in Kenya and Zambia (Table 5). The samples collected included wastewater influent and effluent including the suspended particulate matter, surface water upstream and downstream of the WWTP effluent discharge points, ground water, WWTP sludge and river sediment.

TABLE 4 Physicochemical properties and percentage of consumed APIs excreted in human urine. These can be used to predict the behaviour of the APIs at the WWPs and the receiving water bodies.

API	Mol mass (g/mol)	Excretion as parent compound in urine (%)	S	H	pKa	Log K_{ow}	k_{biol}
Sulfamethexazole	253.3	15-25	610	$2.69x10^{-11}$	1.6–5.7	0.89	0.19–0.2
Doxycycline		70					
Tetracycline	444.4	80–90	3.3, 7.68, 9.69		3.3		
Trimethoprim	290.3	80–90	400	$9.89x10^{-13}$	7.12	0.91	0.05–0.09
Ciprofloxacin	331.3	80	$3 x10^{4}$		6.1,-8.6	-0.28	0.55
Ofloxacin	361.4				6.05,8.2	-0.39	
Norfloxacin	319.3	60	$1.8x10^{5}$	$6.8x10^{-13}$	6.3–8.7	-1.03	0.01 – 0.3
Amoxicillin	365.4	60–80	3430	$2.49x10^{-21}$	3.2	0.87	1.33
Rifampicin	822.9				1.7, 7.9	4.24	
Erythromycin	733.9		1.4	2.2×10^{-27}	8.9	2.5–3.0	
Lamivudine	229.3	70	$7 x 10^{4}$		4.3	-1.4	
Nevirapine	266.3	2.7	$7 x 10^{-4}$	$3.3 x 10^{-17}$	2.8	2.5	0.03
Zidovudine	267.3	15–20	$2 x 10^{4}$		9.86	0.05	

S = solubility in water (mg l^{-1}); *H* = Henry coefficient (1 g m^{-3} air/1 g m^{-3} wastewater); *pKa* = Dissociation constant; K_{ow} = octanol-water partition coefficient; k_{biol} = pseudo first-order degradation constant (1 g^{-1} SS day^{-1}) (Ngumba *et al.* 2020, Ohoro *et al.* 2022).

TABLE 5 Sample type and collection sites for this study. SD = sediments; SL = sludge; GW = groundwater; SSU = source separated urine; SW = surface water; WWE = wastewater effluent/treated wastewater; WWI = wastewater influent/untreated WW; WSPs = waste stabilization ponds/ lagoons, WWTP = wastewater treatment plant, composite = 12–24 hour composite sample.

Country	Location	Sample type	No of samples	Sampling time
Kenya	Nyeri (WWTP; WSP) (I, II)	SL, SW grab, SD WWI/WWE composite	36	Jan & Sep 2019
	Machakos (WSP) (I, II)	SW-grab, SD, WWI/WWE composite	24	Jan & Sep 2019
	Meru (WSP)	SW-grab WWI/WWE composite	8	Jan & Sep 2019
Zambia	Lusaka (WWTP)(III)	WWI/WWE grab	4	Nov 2022; Mar 2023
	Chunga River (III)	SW grab	4	Nov 2022; Mar 2023
	Lusaka (FSM) (III)	SL grab	4	Nov 2022
	Chunga (III)	SSU grab	6	Feb-2023

3.3 Sample treatment and analysis

The environmental samples were collected in clean plastic bottles, transported in cooler boxes to the laboratory, and stored at 4 ^{o}C awaiting further processing within 7 days. 200 ml of 24 hr composite sample from the two WWTPs and four WSPs was used, while 500 ml of surface water and ground water was used for analysis. Tandem filtration of samples through 47 mm GF/D (2.7 µm) and GF/F (0.7 µm) was done to clarify the samples and capture the suspended particulate matter. Clean-up and API extraction was done using an optimized solid phase extraction (SPE) protocol (Ngumba *et al.* 2016a). Extraction of target compounds from suspended particulate matter (SPM), sludge and sediments was done using ultrasonic assisted extraction based on a method published by Al-Khazrajy, Omar S. A. and Alistair 2017, with modifications. Isotopically labelled standards were used as the internal standards for the experiments.

A Waters Alliance 2975 liquid chromatograph (LC, Milford, MA, USA) was used for separation fitted with Xbridge™ (3.5 µm x 2.1 mm x 100 mm) C18 reversed-phase column with Vanguard® (2.1mm x 5mm) pre-column was used. Gradient elution method was used the solvent systems comprising of ultrapure water and acetonitrile acidified with 0.1 % formic acid. A Quattro micro tandem mass spectrometer (MS/MS) set at positive ionisation mode (ESI) used for analyte detection and quantification.

3.4 Calculated risk assessment for antimicrobial resistance evolution in aquatic environment

The risk of residual APIs to bacteria in the environment was assessed (I, II, III). The risk quotient (RQ) for evolution of antimicrobial resistance selection in aqueous samples was calculated based on the MECs of individual compounds and their aquatic PNEC for resistance selection (RS) (Tran *et al.* 2019). The PNEC for a compound can be different between aquatic and terrestrial environments. Compound-specific $PNEC_{(RS)}$ values used in this study were proposed by (Bengtsson-Palme and Larsson 2016) and factored multiple genera of microorganisms present in the environment. The risk quotient (RQ) was calculated based on Equation 1.

$$RQ = \frac{MEC}{PNEC(RS)} \quad (1)$$

where RQ = risk quotient, MEC = measured environmental concentration in the representative aqueous samples, and $PNEC_{(RS)}$ = compound-specific predicted no-effect concentration for resistance selection in aqueous samples. Interpretation $RQ \geq 1$ = high risk, $1 > RQ \geq 0.1$ = medium risk, and $RQ < 0.1$ = low risk (Hanna *et al.* 2018).

3.5 DNA extraction, gene selection and high-throughput qPCR

The samples collected included wastewater influent and effluent, surface water upstream and downstream of the effluent discharge point, and groundwater from a community borehole (Table 6). Sediments corresponding to the aqueous samples were collected where applicable. Fecal sludge sample from the dewatering point of the Manchinchi fecal sludge management plant was also collected. The samples were preserved in DNA/RNA shield buffer and transported to Finland under room temperature conditions. Genetic material extraction was done at the Department of Biological and Environmental Sciences, University of Jyväskylä, Finland based on protocol provided by QIAGEN DNeasy Powersoil pro kit (Qiagen Sciences, Germantown, MD, USA). DNA quality and concentration were analyzed with a Qubit fluorometer (Thermo Scientific, Wilmington, DE, USA). Gene detection and quantification were performed using qPCR targeting 35 antibiotic resistance genes and mobile genetic elements. The relative abundance of each gene in sample was determined in relation to the 16S rRNA gene.

TABLE 6 Sample collection for the antibiotic resistance gene analysis.

Code	Sample name	Sample type
1RS	Chunga river upstream	Sediment
2RS	Chunga river downstream	Sediment
3WS	Chunga WSP effluent	Sediment
4WS	Chunga WSP influent	Sediment
5WS	Manchinchi WWTP influent	Sediment
6FS	Fecal Sludge Management (FSM) Plant	Sludge
7GW	Borehole water	Ground water
8RW	Chunga river upstream	Surface water
9RW	Chunga river downstream	Surface water
10WW	Chunga WSP effluent	Wastewater
11WW	Chunga WSP influent	Wastewater
12WW	Manchichi WWTP influent	Wastewater

RS = river sediment, WS = wastewater sediment, FS = fecal sludge, RW= river water, WW = wastewater.

Various genes of interest that confer resistance to various classes of antibiotics were selected for screening (Table 7). Genes selected for quantification in this study included those that confer resistance to aminoglycoside (1), beta lactams (10), multidrug resistant (1), macrolide-lincosamide-streptogramin-B (3), quinolone (2), sulfonamides (2), tetracycline (5), trimethoprim (2), vancomycin (2), phenicol (2) and others (5). Two integrons (2) were also included. Furthermore, genes that confer

resistance to last resort antibiotics, such as colistin resistance genes *(mcr1, mcr1_1,)*, and vancomycin resistance genes *(vanA, vanB_1)* were selected.

TABLE 7 List of the selected genes for analysis in this study.

No.	Gene	Target antibiotics	No.	Gene	Target antibiotics
1	aac(3)-iid_iia	Aminoglycoside	18	qnrA	Quinolone
2	blaCMY_1	Beta Lactam	19	qnrB_2	Quinolone
3	blaCTX-M	Beta Lactam	20	sul1_1	Sulfonamide
4	blaIMI	Beta Lactam	21	sul4	Sulfonamide
5	blaKPC	Beta Lactam	22	tetA_2	Tetracycline
6	blaNDM	Beta Lactam	23	tetA/B_1	Tetracycline
7	blaOXY	Beta Lactam	24	tetG	Tetracycline
8	blaSFO	Beta Lactam	25	tetW	Tetracycline
9	blaTEM	Beta Lactam	26	tetX	Tetracycline
10	blaVIM	Beta Lactam	27	dfrA1	Trimethoprim
11	penA	Beta Lactam	28	dfrB	Trimethoprim
12	intI1_1	Integrons	29	vanA	Vancomycin
13	intI1_2	Integrons	30	vanB_1	Vancomycin
14	mexE	MDR	31	arr2	Other
15	ermA	MLSB	32	arr3	Other
16	mphA	MLSB	33	bacA	Other
17	pikR2	MLSB	34	mcr1	Other
			35	mcr1_1	Other

3.6 Adsorption experiments

Adsorption experiments were done to determine adsorption capacity and kinetics of selected API mixtures in SSU using different activated carbons (IV). The urine used in this experiment was collected from healthy unmedicated individuals. The collected urine was stored in room temperature for 21 days to guarantee the natural hydrolysis to occur whereby the pH of the urine changes from slightly acidic to basic due to the formation of ammonia (Cook *et al.* 2007). The resulting pH measurement was done followed by spiking with the target model compounds with varying physicochemical properties (SMX, TMP, 3TC and NVP) at concentrations ranges corresponding to those previously measured SSU collected in Lusaka, Zambia. Four different experimental wood and peat based powdered activated carbons were used as adsorbents. The effect of urine matrix on adsorption capacity was tested by carrying out the experiments in spiked ultrapure water Milli-Q (MQ) and in real human urine. The removal of the spiked compounds was monitored for 24 hours at intervals of 15 minutes for the first hour, 30 minutes for the next three hours and hourly for six hours and thereafter left overnight. Samples collected at various time intervals were processed and analysed using SPE-LC-ESI-MS/MS method as described by (Ngumba *et al.* 2016a). The control sample consisted of human urine without adsorbent and underwent the same procedures and analysis. The analyte concentration in the filtrates were measured for all the treated samples and control samples. Adsorption capacity (q_e) expressed in milligrams of API adsorbed per gram of adsorbent (mg g^{-1})

was determined at equilibrium (Equation 2). The adsorption equilibrium concentration was deduced to occur at a time when no significant change occurred in the concentration of the solution (Otieno *et al.* 2021). The percentage removal of the target APIs was also determined.

$$q_e = \frac{(C_0 - C_e)V}{W} \quad (2)$$

where q_e is the adsorption capacity C_o and C_e are the concentrations of solute at the initial time and at equilibrium (mg l^{-1}), respectively, V is the volume of solution (l), and W the mass of adsorbent (g)

4 RESULTS AND DISCUSSION

4.1 Occurrence and phase partitioning of APIs

4.1.1 Occurrence in the aqueous phase

Occurrence of the selected APIs in the different stages of the urban hydrological cycle was studied. Aqueous samples included wastewater influent and effluent, surface water and ground water. Measured concentrations of selected antimicrobials in wastewaters ranged between < LOQ to 49.3 μg l^{-1} (I), 1.4 to 956.4 μg l^{-1} (II); and < LOQ to 1.8 μg l^{-1} (III) respectively. API residues in the surface waters ranged between <0.1 to 56.6 μg l^{-1} (I), 1.1 to 228.3 μg l^{-1} (II), and < LOQ to 1.78 μg l^{-1} (III). The concentration of APIs measured in these studies are summarized in Table 8 and compared with other reported values in literature. TMP and SMX were consistently abundant antibiotics in the aqueous phase. SMX was the most abundant antibiotic in the wastewaters with measured concentrations of 49.3 μg l^{-1} and 94.2 μg l^{-1} in WWTP influent samples (I, II), in surface water upstream and downstream of the effluent discharge point with 56.6 μg l^{-1} (I) and 142.6 μg l^{-1} (II) respectively. TMP and SMX are often administered as combination (co-trimoxazole) because of their synergistic treatment effects to a wide range of infections, including prophylactic treatment in tuberculosis and HIV/AIDS patients. For the antiviral category, lamivudine (3TC) was most abundant with measured concentration of up to 228.3 μg l^{-1} in surface water, downstream of the effluent discharge point (II). 3TC is among the first-line ARVDs recommended for antiretroviral therapy (WHO 2022).

Higher concentrations of some APIs were frequently measured in the WWTP effluent relative to influent, mostly in the treatment systems without proper sludge collection and removal. For instance, the treatment system WWTP 2 (I) which employed waste stabilization ponds had CIP, NOR and AMO measured in higher concentrations in effluent relative to influent. Furthermore, SMX occurred at higher concentrations in the surface water (0.7 μg l^{-1}) compared to influent (0.4 μg l^{-1}) and effluent (0.5 μg l^{-1}) (III). This increase could be attributable to the characteristics of APIs, such as accumulation in the treatment system, deconjugation and retransformation of metabolites as well as resuspension of sorbed compounds. This

study did not focus on measurement of API transformational products (TPs), but their potential influence on the amount of parent compound measured at the effluent relative to the influence was suspected. For instance, deconjugation and retransformation processes for TPs of SMX back to parent compound within the wastewater treatment processes is documented in literature (Radke *et al.* 2009, Polesel *et al.* 2016, Nguyen *et al.* 2018, Li *et al.* 2019, Brown *et al.* 2020, Castaño-Trias *et al.* 2023).

Surface waters receiving the discharged effluent had occasional higher MECs compared to the corresponding effluent. The phenomenon was attributed to direct release of untreated sanitation products (human excreta and blackwater), with higher concentration of APIs that than that emitted from treatment systems, directly to the receiving water bodies. The discharge would especially emanate from informal settlements in the peri-urban areas of the city such as Lusaka with high population density and relatively higher disease burden coupled with poorly managed sanitation services. Furthermore, the receiving waters have perennial low volumes except in rainy seasons, for instance in Machakos, Kenya because of the numerous direct withdrawals of the water for domestic use and subsistence vegetable farming along the riverbanks.

Occurrence of antibiotics and ARVDs used in ART at similar or occasionally higher than other ARVDs in the environment was also noted. For instance, lamivudine (3TC) was measured at 847 μg l^{-1}, 219 μg l^{-1} and 228 μg l^{-1} in wastewater effluent, and surface water (upstream and downstream) respectively (II). These concentrations reflect the increased uptake (83 %) of antiretroviral therapy in selected sub-Saharan countries compared to other parts of the world (Adeola and Forbes 2022, UNAIDs 2023). The ARVDs residues monitored in this study were part of a continuous monitoring study. The environmental concentrations of ARVDs are of significance because they were previously shown to have ecotoxicological effects on aquatic organisms (Ngumba *et al.* 2016b, Mahaye and Musee 2022). Furthermore, there is rising concern about the emergence of antiviral resistance in the environment and more data to map out the MECs for ARVDs is needed (Nannou *et al.* 2020).

SMX, CIP, SDX, ERY, TMP, ZDV, NVP and 3TC were detected in ground water from Lusaka Zambia at concentration ranging from <LOQ to 0.151 μg l^{-1}(III). SMX and ERY had the highest concentrations of 0.03 and 0.01 respectively while most of the other compounds were below detection limit. Previously, SMX, CIP, TMP, AMO and NVP was detected in the ground water samples in the peri-urban areas of Lusaka at concentrations ranging from 0.14 μg l^{-1} to 0.66 μg l^{-1} (Ngumba *et al.* 2020). The temporal variations are attributed to seasonal changes since the initial sampling (2016) was done on drought conditions and the latter (2022) was done on rainy conditions. Nevertheless, these concentrations were comparable with those reported in other research areas (Table 8) with occasional higher values reported. Presence of APIs in the ground water sources was attributed to the leaching effects due to proximity to the pit latrines and compost pits to these water sources with high water table.

TABLE 8 Concentrations of APIs in environmental samples measured in this study and compared with other concentration values reported in literature. ( ) = standard deviation.

API	Country	Influent (µg l^{-1})	Effluent (µg l^{-1})	Effluent SPM (µg kg^{-1})	Surface water (µg l^{-1})	River sediments (µg kg^{-1})	Groundwater (µg l^{-1})	References
SMX	Kenya Machakos	49.3(2.7)	8.5(0.4)	na	3.4(0.7)	<LOQ	na	I
	Kenya	n.a	94.20	23448 (1959)	142.5(9)	895.56(29)		II
	Zambia-Chunga	0.72	0.36	n.a	0.45	n.a	0.01	III
	Kenya	n.a	3.34	n.a	13.80	n.a	n.a	Ngumba *et al.* (2016a)
	Finland	0.22	0.11	n.a	0.03	na	n.a	Ngumba *et al.* (2016a)
	Zambia	33.30	30.04	n.a	11.80	na	0.66	Ngumba *et al.* (2020)
	China	n.r	n.r		0.03		0.00	Tong *et al.* (2014), Yao *et al.* (2017)
	Sweden	0.67	0.30		n.r		n.r	Lindberg *et al.* (2005)
	Spain	n.r	n.r		n.r		0.07	López-Serna *et al.* (2013)
	USA	n.r	n.r		n.r		0.11	Schaider *et al.* (2014)
	Kenya	54.83	4.09		39.00		0.03	K'oreje *et al.* (2016)
	South Africa	59.28	1.60		8.70		n.r	Matongo *et al.* (2015a), Agunbiade and Moodley (2016)
	India	0.22	0.26		n.r		n.r	Subedi *et al.* (2017)
	Africa	n.r	n.r		2.53		n.r	aus der Beek *et al.* (2016)
CIP	Kenya-Machakos	1.6(0.4)	0.4 (0.3)	n.a	<LOQ	<LOQ	n.a	I
	Kenya Machakos	9.9(1.9)	5.99(1.1)	31117(349)	6(1.3)	290.41(21)	n.a	II
	Zambia-Chunga	0.31	0.08	n.a	0.08	n.a	0.01	III
	Zambia-Chunga	0.74	0.23	n.a	0.54	n.a	0.15	Ngumba *et al.* (2020)

	Spain	n.r	n.r		n.r		0.44	López-Serna *et al.* (2013)
	South Africa	27.10	14.10		14.30		n.r	Agunbiade and Moodley (2016)
	Vietnam	<MDL–3.035	n.r		n.r		n.r	Tran *et al.* (2019)
	Finland	4.23	0.13		0.04		n.r	Vieno *et al.* 2006, 2007)
	Africa	n.r	n.r		0.02		n.r	aus der Beek *et al.* (2016)
NOR	Kenya	2.8	0.8	n.a	<LOQ	1.8	n.a	I
	Kenya	3.9 (0.8)	3.57(0.6)	12617 (712)	5.9(0.9)	327.6 (44)	n.a	II
	Finland	0.24	0.10	n.a	0.05	n.a	n.a	Ngumba *et al.* (2016b)
	China	n.r	n.r		0.28		0.10	Tong *et al.* (2014), Yao *et al.* (2017)
	Spain	n.r	n.r		n.r		0.00	López-Serna *et al.* (2013)
	Australia	2.20	2.50		11.50		n.r	Watkinson *et al.* (2009)
	Sweden	0.17	0.04		n.a		n.r	Lindberg *et al.* (2005)
	France	n.a	n.a		0.16		n.r	Tamtam *et al.* (2008)
	Africa	n.r	n.r		0.08		n.r	aus der Beek *et al.* (2016)
TMP	Kenya	4.8	0.5	n.a	n.a	1.8	n.a	I
	Kenya	11.27(2)	158 (12)	3080 (845)	4.36(0.4)	90.03(6.3)	n.a	II
	Zambia	1.36	0.63	n.a	0.6	n.a	0.002	III
	Finland	0.57	0.54	n.a	0.02	n.a	n.a	Ngumba *et al.* (2016a)
	Zambia	32.67	1.77	n.a	2.41	n.a	0.14	Ngumba *et al.* (2016b)
	China	n.r	n.r		0.02		0.01	Tong *et al.* (2014)
	Spain	n.r	n.r		n.r		0.01	López-Serna *et al.* (2013)
	Kenya	72.85	0.15		7.00		0.01	K'oreje *et al.* (2016)
	USA	n.a	n.a		0.02		0.00	McEachran *et al.* (2016)
	South Africa	0.01	0.16		0.87		n.r	Matongo *et al.* (2015a, b)
	Mozambique	n.r	n.r		6.22		n.r	Segura *et al.* (2015)
	Africa	n.r	n.r		0.99		n.r	aus der Beek *et al.* (2016)

DOX	Kenya-machakos	2.7	1.4	n.a	0.3	8.2	n.a	I
	Kenya-nyeri	<LOQ	0.7	n.a	<LOQ	<LOD	n.a	I
	Finland	0.054	0.016	n.a	n.d	n.a	n.a	Ngumba *et al.* (2016a)
	Zambia	4.5	5.28	n.a	3.26	n.a		Ngumba *et al.* (2020)
	China	n.r	n.r		0.07		0.06	Tong *et al.* (2014)
	Spain	n.r	n.r		n.r		0.19	López-Serna *et al.* (2013)
	Australia	0.65	0.15		0.40		n.r	Watkinson *et al.* (2009)
	Ghana	n.a	n.a		0.01		n.r	Segura *et al.* (2015)
	Sweden	2.48	0.88		n.r		n.r	Lindberg *et al.* (2005)
TET	Zambia	<LOQ	<LOQ				<LOQ	III
	Finland	0.04	0.03	n.a	nd	n.a		Ngumba *et al.* (2016a)
	Zambia	0.22	4.59	n.a	4.22	n.a	nd	Ngumba *et al.* (2020)
	China	n.a	n.a		0.10		0.03	Tong *et al.* (2014), Yao *et al.* (2017)
	South Africa	5.68	1.70		2.80		n.r	Agunbiade and Moodley (2016)
	Spain	n.r	n.r		n.r		56.30	López-Serna *et al.* (2013)
	Ghana	n.r	n.r		465.00		n.r	Segura *et al.* (2015)
	Kenya	n.r	n.r		434.00		n.r	Segura *et al.* (2015)
	Belgium	1.66	n.d		n.r		n.r	Vergeynst *et al.* (2015)
AMO	Kenya Machakos	4.6	1.6		0.05	4.6	n.a	I
	Kenya-Nyeri	0.2	0.9	n.a	<LOD	<LOD	n.a	I
	Kenya-Nyeri	0.70	1.24		0.30	43.8	n.a	I
	Kenya Meru	1.58	1.4	n.a	<LOD	7.8	n.a	I
	Finland	0.116	0.069	n.a	n.d	n.a	n.a	Ngumba *et al.* (2016a)
	Zambia	3.27	5.58	n.a	3.41	n.a	0.66	Ngumba *et al.* (2020)
	Australia	6.94	0.05		0.20		n.r	Watkinson *et al.* (2009)

	United Kingdom	n.r	n.r		0.25		n.r	Kasprzyk-Hordern *et al.* (2007)
	Germany	1.27	0.19		n.r		n.r	Rossmann *et al.* (2014)
	Vietnam	<MDL–20.6	n.r		n.r		n.r	Tran *et al.* (2018)
3TC	Kenya Machakos		847.1	69681	228.3	107	n.a	II
	Kenya	60.68	31.07		167.00		n.r	K'oreje *et al.* (2016)
	South Africa	n.r	n.r		0.13		n.r	Wood *et al.* (2015)
	Germany	0.72	n.d		n.d		n.r	Prasse *et al.* (2010)
ZDV	Kenya-Machakos		1.4	3336	1.1	118	n.a	II
	Kenya	20.13	0.11		17.00		0.03	K'oreje *et al.* (2016)
	Germany	0.38	0.56		0.17		n.r	Prasse *et al.* (2010)
NVP	Kenya-Machakos		9.5	3214	2.3	101	n.a	II
	Kenya-Kisumu	3.30	2.08		5.62		1.60	K'oreje *et al.* (2016)
	South Africa	2.10	0.35		1.48		n.r	Wood *et al.* (2015), Mashiane M (2015)
	Germany	0.02	0.03		0.01		n.r	Prasse *et al.* (2010)

n.r = not reported; n.d = not detected, n.a = not analysed <MDL = below the method detection limit, n.a = not analysed; <LOQ= concentration lower than limit of quantification; n.d = not detected

Generally, the MECs of selected APIs in Kenyan and Zambian samples frequently occurred at concentrations 10 to 1000-fold higher relative to literature values from Finland and other high-income countries. The risk associated with APIs in the environment is concerning, and getting more attention from governments, policy makers/shapers and legislative institutions. In the European Union, new proposals for legislation that will make risk assessment and risk mitigation measures for human pharmaceuticals mandatory are already at various stages of advancement (Moermond *et al.* 2023, Gildemeister *et al.* 2023). These proposals are aimed at making environmental protection mandatory and connect the legislative framework for pharmaceuticals and the environments. Some of the proposed APIs for inclusion in the EU watchlist for stricter regulation were included in this study. For instance, MECs for CIP, SMX and TMP (I, II) occurred above the concentration values proposed for regulation and inclusion in the watchlist in the proposed EU water framework directive; national environmental quality standards and other independent studies (Bengtsson-Palme and Larsson 2016, Ågerstrand *et al.* 2023). These compounds are candidates in the EU watchlist compounds based on their potential health effects.

The divergence in the occurrence patterns of API residues in the representative LMICs and the HICs potentially reflects the status of disease burden in the society, pharmaceutical consumption patterns, sanitation systems, waste management systems and wastewater treatment. Occurrence of APIs in the environment is correlated to the medicine consumption patterns (Boulard *et al.* 2020). Concentration values of selected APIs in global South have been reported (Table 8) at the the same order of magnitude, or considerably higher than those reported in the global North. The high concentration of APIs in environment in global South potentially means that the local population are in a close contact with highly contaminated water sources, a cause for concern for the wellbeing of the ecosystem.

4.1.2 Occurrence of APIs in SPM, sediments, and sludge

The concentrations of selected APIs in SPM, sediment and sludge samples was determined. The SPM had concentrations ranging between < LOD to 82.3 mg kg^{-1}. In the river sediments concentrations ranged between <LOD to 0.47 mg kg^{-1} (I), and 0.11 to 4.125 mg kg^{-1} (II) respectively. The sludge samples had concentrations occurring between < LOQ to 31.6 mg kg^{-1}. NOR and CIP showed the highest concentrations in both the SPM sediment and sludge, which was attributed to their specific characteristics, such as their zwitterionic character in the wastewater pH conditions. This renders CIP and NOR to sludge adsorption by hydrophobic and electrostatic interactions (Lindberg *et al.* 2006, Verlicchi *et al.* 2012). AMO was the least detected in the SPM, sludge, and sediment samples, and was also infrequently detected in the aqueous phases. The API concentration in the SPM varied based on the specific compound. Furthermore, lack of sludge removal or recycle mechanisms in all the sampled WWTPs except WWTP3 (I) could potentially influence the measured concentration of APIs due to resuspension of adsorbed APIs.

Higher concentrations of APIs were detected in the SPM phase and the sludge, relative to the aqueous samples and the river sediments in some sampling sites (II).

Therefore, the SPM is potentially an important route for emission of APIs to environment in some of the sampled WWTPs. The APIs bound to SPM phase potentially represented the unremoved part of the influent suspended matter and escaping sludge that is formed in the WWTP where there was no removal or recycle mechanism for the sludge. The mechanism used to bind the API residues to SPM is complex and poorly understood but is mostly attributable to sorption. The type and nature of sorption that occurs depends on both the properties of API compounds, such as charge, hydrophobicity; as well as the SPM matrix such as clay content and amount of organic matter (Boulard *et al.* 2020, Ledieu *et al.* 2021). The removal efficiency which represented the extent of abatement of the selected API the wastewater treatment process was evaluated. The overall removal factored both the aqueous and SPM phases and ranged between 69 % and –89 %. Individual APIs were removed differently based on the type of wastewater treatment (I, II). Therefore, it was concluded that in calculation of API removal efficiency from treatment system the contribution of all phases need consideration.

Quantification of APIs in aqueous matrices is a focal point in numerous investigations, with insufficient attention often given to their presence within solid particulate matter and river sediments (Castaño-Trias *et al.* 2023). Our current study registered the notable capacity of the solid particulate matter (SPM) phase to enhance API emissions from wastewater treatment plants (WWTPs), owing to the consistently measured higher concentrations of pharmaceutical residues compared to the aqueous phases. The mass emission of APIs to the environment from the WWTP can therefore be biased by overlooking the contribution of the SPM phase (Ledieu *et al.* 2021). Consequently, it becomes evident that the SPM phase assumes a critical role in the context of pharmaceutical emissions within unconventional wastewater treatment plants and lagoon systems (II). Furthermore, the downstream impact of effluent SPM on river sediments is notable, as manifested by higher concentrations of pharmaceutical residues downstream from the effluent discharge point in contrast to upstream samples (I). This underscores the significance of accounting for the SPM phase when assessing the emission dynamics of pharmaceuticals in non-conventional wastewater treatment systems.

4.2 Calculated risk quotient for antibiotic resistance evolution in aquatic environment

Development of antibiotic resistance in the environment is a complex issue in which API residues are a contributing factor. According to literature, measured environmental concentrations of APIs can be used to predict the risk quotient for antibiotic resistance evolution in that environment (Bengtsson-Palme and Larsson 2016). Yet, the extent to which environmental pollution from antibiotics contributes to

resistance development remains unclear. However, the observed concentrations of environmental antibiotics in this thesis often surpass concentration predicted to favor the selection of resistance (Bürgmann *et al.* 2018, Larsson and Flach 2021, Ågerstrand *et al.* 2023). Currently, many of the resistance factors faced at the clinic originate from environmental bacteria (Ebmeyer *et al.* 2021, Ågerstrand *et al.* 2023). In this thesis, the aquatic risk quotients (*RQ*s) for antibiotic resistance selection in Kenya and Zambia were calculated based on the compound-specific $PNEC_{(RS)}$ concentration values as proposed by (Bengtsson-Palme and Larsson 2016). Previously measured MECs were used to calculate the $PNEC_{(RS)}$ for environmental samples collected from Finland (Ngumba *et al.* 2016a). The proposed $PNEC_{(RS)}$ included NOR (0.5 µg l^{-1}), CIP (0.064 µg l^{-1}), TMP (0.5 µg l^{-1}), SMX (16 µg l^{-1}), DOX (µg l^{-1}), and AMO (0.25 µg l^{-1}). The mean concentrations reported in this thesis for the recipient waters included SMX (<0.01 to 8.9 µg l^{-1}) CIP (0.78 to 9.4 µg l^{-1}) NOR (0.18 to 3.2 µg l^{-1}) and TMP (0.03 to 8.7 µg l^{-1}).

Several pharmaceutical concentrations observed in the surface waters have raised significant concerns as their calculated risk quotients surpass the proposed threshold values for development of antimicrobial resistance. The *RQ*s exhibited a considerable range from 0.001 to 93.5 (Figure 2, with *RQ* >1 indicating high risk), signifying low to high risk for evolution of antimicrobial resistance in aquatic environments within the analyzed samples. Among the analyzed antibiotics, ciprofloxacin (CIP) demonstrated the highest *RQ* of 93.8, 43,9, 8.4, 7.9 followed by norfloxacin (NOR) with an *RQ* of 11.8, 3.2 and amoxicillin (AMO) with an *RQ* of 13.6 in surface water samples collected from Kenya. Zambian samples had *RQ* values ranging from 0.22 to 14 (Table 9) signifying medium to high risk. In contrast, previously analyzed environmental samples from Finland (Ngumba *et al.* 2016a) had low to medium risk with risk quotients ranging between 0.001 and 0.7.

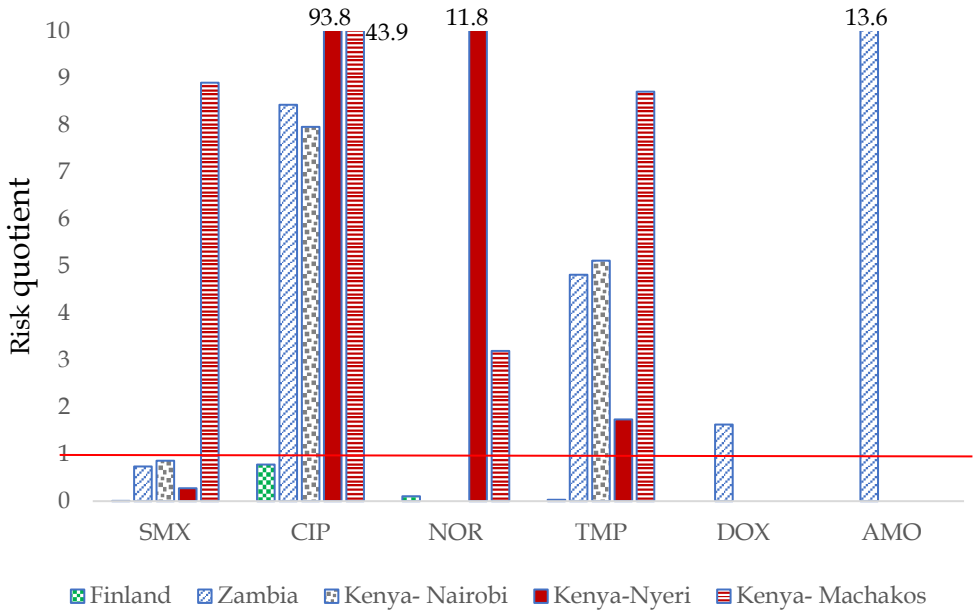

FIGURE 2 Calculated risk for evolution of Antibiotic resistance in recipient surface waters. *RQ* ≥*1* = high risk, *1* >*RQ* ≥*0.1* = medium risk, and *RQ* < *0.1* = low risk (Bengtsson-Palme and Larsson 2016, Hanna *et al.* 2018).

In the study of Lusaka Zambia, the MECs of antibiotic residues were measured in samples collected in 2022 at relatively lower concentrations as compared to a previous study done in 2016 (III). This could possibly be due to seasonal changes in weather conditions such as rain and drought during the sampling periods. Furthermore, lower MECs can be influenced by changes in medication consumption patterns resulting from reduced disease burden, or increased access to improved sanitation systems in the sampling areas. Nevertheless, medium to high risk for evolution of antibiotic resistance was calculated in these samples (Table 9).

It should be noted that the antibiotic resistance in the environment can persist longer even after elimination of selective pressure factors, due to horizontal gene transfer (Larsson and Flach 2021). Therefore, the MECs may not reveal underlying *RQ* or selection pressures happening in longer time scales.

TABLE 9 Temporal variation of antibiotic residues and risk of antibiotic resistance evolution in surface waters of Lusaka Zambia in 2016 and 2023.

Compound	Previous study	Calculated Risk quotient	PNEC ($\mu g\ l^{-1}$) [b]	Current study	Calculated Risk quotient
	2016[a]			2022 (III)	
Amoxicillin	2.5 - 3.4	10-13.6 (High risk)	0.25	<LOQ	-
Trimethoprim	0.5 - 2.4	1-5 (High risk)	0.5	0.01 - 1.36	0.02-2.72 (low -high risk)
Oxytetracycline	na	-	0.5	<LOQ	-
Ofloxacin	na	-	0.5	<LOD – 0.05	<0.01-0.1 (low -medium risk)
Tetracycline	0.22 - 4.6	0.22 - 4.6	1	<LOQ	-
Ciprofloxacin	0.23 - 0.74	3.6 - 11.6 (High risk)	0.064	<LOD – 0.31	0-4.8 (low -high risk)
Sulfamethoxazole	7.8 - 33.3	0.49-2.1 (medium - High risk)	16	0.01 - 0.72	0.001-0.05 (low risk)
Sulfamethoxypyrazine	na	-	nr	<LOQ	-
Sulfadoxine	na	-	nr	0.074 - 0.83	-
Erythromycin	na	-	8	0.030 - 1.79	<0.1 - 0.22 (low -medium risk)
Rifampicin	na	-	0.5	<LOQ - 0.65	<0.1 – 1.3 (low -high risk)

na = Not analyzed, <LOQ = Below limit of quantification

4.3 Presence of antibiotic resistance genes in the environment (III)

In recent times, there has been a growing interest to study the role of environment in the evolution of antibiotic-resistant pathogens and their transmission pathways. The reported evidence in literature shows the environment is an important vector in resistance evolution and transmission mechanisms. The WWTPs have been identified as hotspots for development and proliferation of ARBs and ARGs whereas the recipient water bodies act as reservoirs and dissemination routes (Karkman *et al.* 2018, Kim and Cha 2021)

In study III, genes that confer resistance to multiple classes of antibiotics, including last resort antimicrobials were detected in the environmental samples from Lusaka, Zambia. This is an issue of concern because of the possible mobilization to pathogenic bacteria and possible spread to humans and animals. The number of genes detected per sample out of the selected 33 resistant genes ranged between 29 to 32 (83–87 %), suggesting a relatively high detection rate. The gene abundance relative to 16S rRNA ranged from 1.3×10^{-8} to 5.2×10^{-1} (Figure 3) with the ARGs that confer resistance to beta-lactams, sulfonamides, trimethoprim and last resort antibiotic rifamycin showing higher relative abundance values.

The impact of effluent wastewater on surface water sediments downstream of the discharge point was noted, with almost similar relative gene density detected in the surface waters and river sediments as the effluent wastewater. Accumulation of ARGs in sediments may depend on factors such as deposition rates, sorption, enzymatic degradation, and sediment type (Liu *et al.* 2020, Mcinnes *et al.* 2021). Furthermore, clay particles provide further protection to bound DNA adsorbed to soil particles (Deshpande and Fahrenfeld 2022). No ARGs were detected in the groundwater, which is the main drinking water source in that locality.

Measured environmental concentrations in this study could be useful indicators for assessment of environmental risk for evolution of ABR, especially in the absence of the infrastructure needed for analysis of environmental resistomes. Furthermore, the measured concentrations can be used to map the possible hotspots based on the *RQ* values.

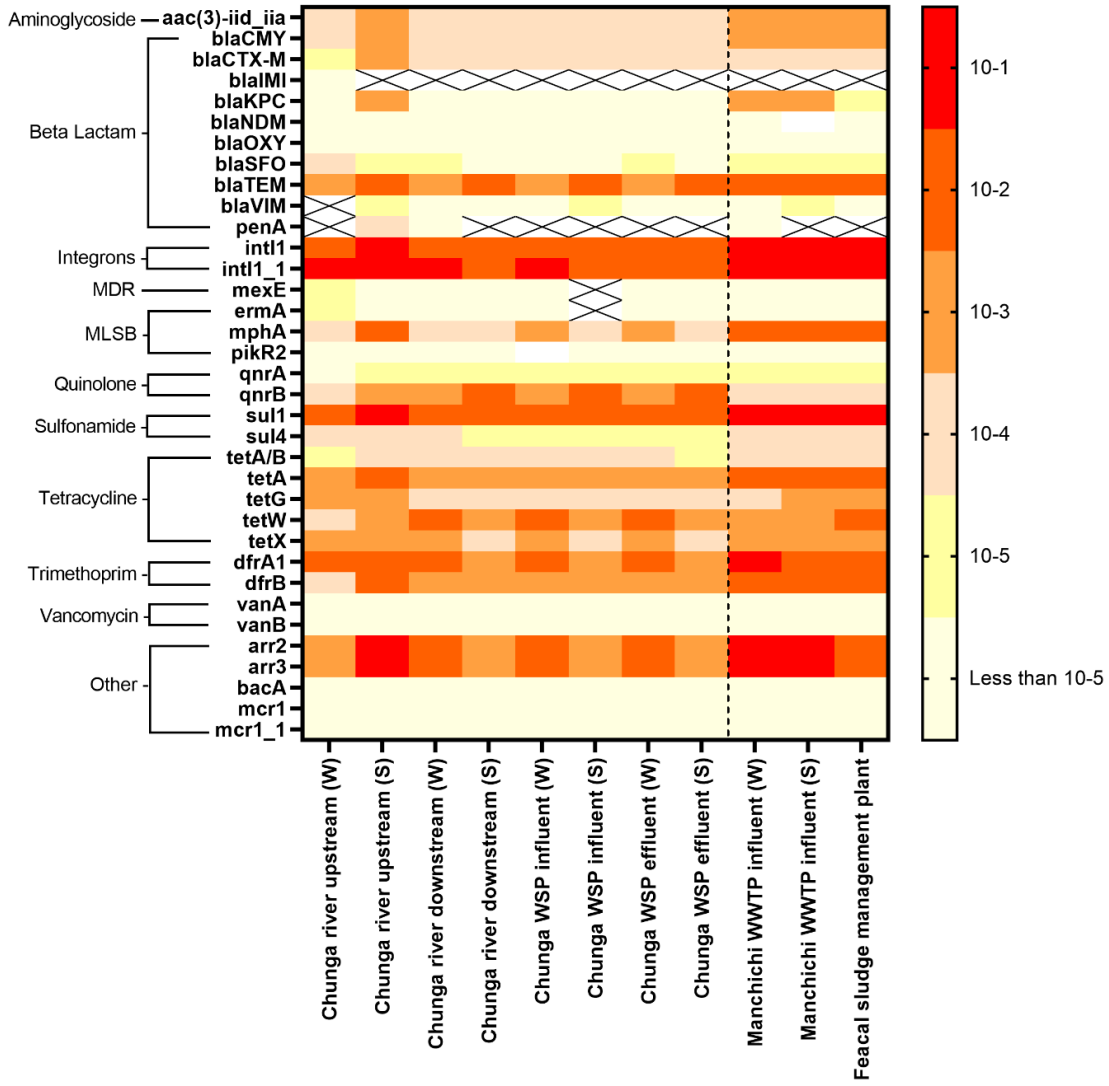

FIGURE 3 Heatmap of the antibiotic resistance gene abundance relative to the 16S rRNA gene in DNA samples extracted from water surface (W) and sediment (S) in Lusaka, Zambia. Gene relative abundance corresponding to values between 10^{-8} to 10^{-5} is represented by light yellow and 10^{-1} was represented by red. Undetected gene expression is indicated by "X".

4.4 Removal of APIs using activated carbon (IV)

Removal of four active pharmaceutical ingredients (APIs) from human urine using wood and peat based activated carbons (RD1, RD2, RD3 and RD4) was investigated.

This was done to assess the feasibility of using relatively simple technology to remove APIs from SSU. The urine samples were spiked with four model compounds with varying physico-chemical properties such as molecular properties, solubilities, acid dissociation constant among others. These included sulfamethoxazole, nevirapine, trimethoprim and lamivudine (SMX, NVP, TMP, and 3TC), each at 500 µg l^{-1} concentration. This concentration was based on relevant concentrations previously measured by a study in the research group which measured concentrations of selected APIs in real urine collected in source separating urine collected in urine diverting dry toilets (UDDTs) in Lusaka, Zambia (Ngumba *et al.* 2020). Effect of adsorbent dosage, contact time, and initial concentration of the selected pharmaceutical residues were studied.

The maximum adsorption capacities of activated carbon for the APIs in ultrapure water (MQ) and real urine matrix ranged between 10–200 mg g^{-1} and 0.75–16.6 mg g^{-1} respectively. The results showed that organic matter measured as DOC (1500 mg l^{-1}) and total nitrogen (2570 mg l^{-1}) concentrations in urine had an approximate 10-fold decrease in adsorption capacity compared to MQ water. However, removal efficiencies were >99 % after 24 hrs. TMP, NVP and 3TC were best described using Langmuir model, while Freundlich model was best for SMX, respectively. Experiment conducted using the wood based RD2 carbon (Figure 4) proved that approximately 18 g l^{-1} of RD2 carbon is sufficient to eliminate the spiked APIs (<1–15 mg l^{-1}) to below quantifiable limits in real urine in 12 hrs. This was sufficient to remove even the highest API concentration (TMP at 12800 µg l^{-1}) previously measured in real urine (Ngumba 2020). The RD activated carbons used in this study could be effective adsorbent for the removal of APIs from urine, despite the complex matrix that occurred 2–3 orders of magnitude more than the APIs. The required dose for removal of APIs from SSU is realistic for practical purposes. For instance, it has been reported that patients under medication are a point source of API residues for that period of medication and sometimes slightly longer (Viskari *et al.* 2018). Concentration of the APIs in the human urine is extremely high relative to concentration levels measured in the wastewaters and surface waters. Therefore, controlling the emission of the pharmaceutical residues from individuals and household could be an effective first control barrier using targeted tools and technologies that are easily applicable at the individual level. It offers a starting point for further development of simple and rapid technology with absorbents made of inexpensive and locally available materials.

Removal of APIs from urine using carbon materials is favored because of ability to eliminate APIs without significant reduction in beneficial nutrients present (Köpping *et al.* 2020, Duygan *et al.* 2021). The adoption of urine diverting technology can be accelerated by the opportunity of risk reduction of micropollutants, such as antibiotics in urine that can cause spread of AMR bacteria and genes in environment. This could be a proof of concept that treatment of low volumes, but high concentrations is less complicated. With no centralized collection and treatment, the on-site urine diversion and treatment could offer an alternative control method for API mass flow to environment.

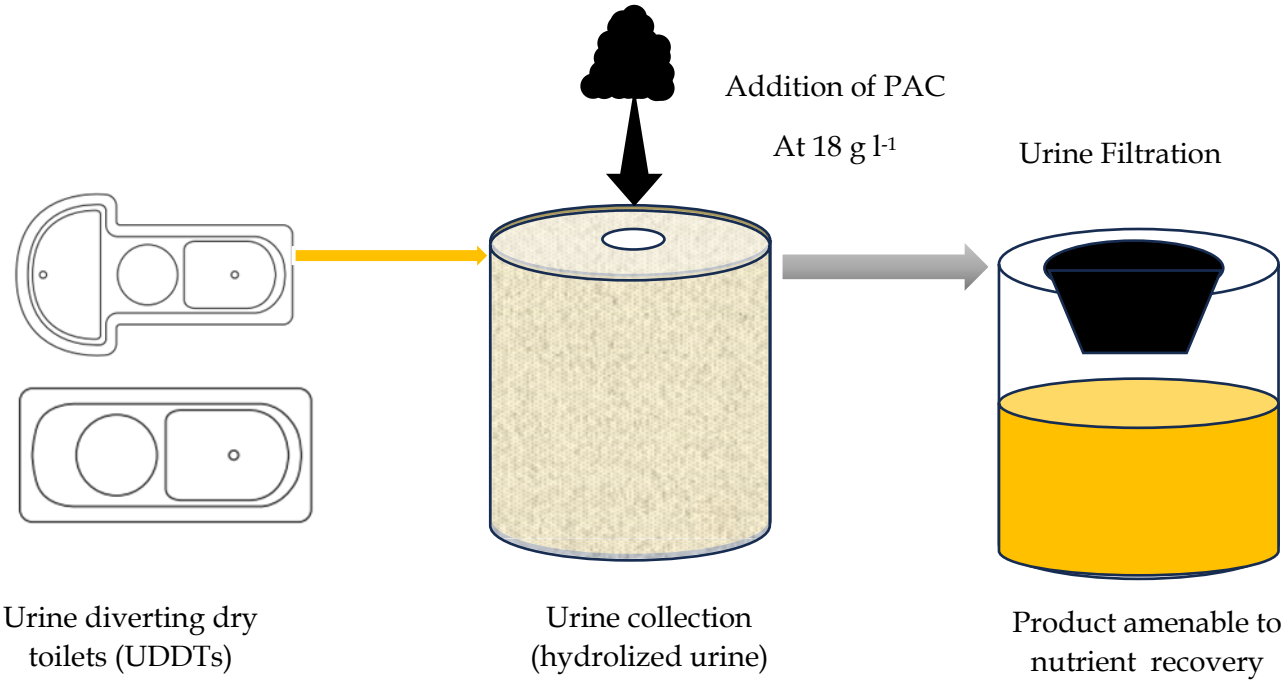

FIGURE 4 Illustration of a possible setup for onsite treatment for the removal of APIs from urine diverted at the household level using powdered activated carbon.

5 CONCLUSIONS

Based on the objectives of this thesis, the following conclusions can be drawn:

i. End of pipe technologies, such as centralized WWTPs are unrealistic approach in low- and medium-income countries to combating continuous flow of APIs into the receiving waters because they are incapable of complete removal of antibiotic residues (I, II).
ii. The suspended particulate matter is a potential important phase for consideration in the determination of API environmental concentrations (II).
iii. Environmental contamination of water bodies by APIs and ARGs is disproportionately higher in Kenya and Zambia compared to high-income countries potentially due to limited sanitation coverage, ineffective and nonfunctioning wastewater collection and treatment systems (I, II, III). Furthermore, the measured concentrations can be used to map the possible hotspots based on the *RQ* values.
iv. Use of cheap and robust point-of-source technologies, such as adsorption of micropollutants on activated carbon materials offers a promising starting point for treatment of source separated urine. This is because of the efficacy in removal of APIs from urine (IV).

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Lääkeaineiden esiintyminen ympäristössä voi aiheuttaa ympäristölle ja terveydelle riskejä. Varsinkin antibiootit edesauttavat antibioottiresistenssin syntymistä ympäristössä jo pieninä pitoisuuksina ja täten pahentavat antibiooteille resistenttien bakteerikantojen syntymistä. Bakteerien antibioottiresistenssi on nopeasti kasvava ongelma ja se vaikeuttaa merkitsevästi infektioiden ehkäisyä ja hoitoa ympäri maailman.

Tässä väitöskirjassa mitattiin valittujen antibioottien sekä antiretroviraalisten lääkeaineiden pitoisuuksia Keniassa ja Sambiassa käsittelemättömistä ja käsitellyistä jätevesistä, vastaanottajavesistöistä sekä pohjavedestä. Erityistä huomiota kiinnitettiin antibioottien jakaantumiseen nestefaasin ja kiintoaineen välillä jätevedessä, lietteessä sekä vastaanottajavesistöjen vesifaasissa sekä sedimentissä. Lääkeaineiden pitoisuuksia mitattiin myös kohteista, jossa ei ole järjestettyä jätevesien keräilyä eikä käsittelyä, vaan lääkeaineet päätyivät muun orgaanisen aineen, ravinteiden sekä patogeenien mukana mukana suoraan ympäristöön. Lääkeaineiden pitoisuustasoja verrattiin vastaaviin pitoisuuksiin Suomessa. Sambian näytteistä analysoitiin myös 35 antibioottiresistenssiin liittyvän geenin suhteellinen osuus bakteeriyhteisössä kvantitatiivisen PCR:n avulla.

Mitatut lääkeainepitoisuudet olivat tutkituissa kohteissa Keniassa ja Sambiassa huomattavasti paljon korkeampia kuin Suomessa ja muissa korkean kehitystason maissa. Käsitelemätön jätevesi sisälsi korkeita yhteispitoisuuksia tutkittuja lääkeaineita, maksimissaan 49,3 $\mu g\ l^{-1}$. Käsitellyssä jätevedessä saattoi esiintyä jopa käsittelemätöntä jätevettä korkeampia pitoisuuksia, 956,5 $\mu g\ l^{-1}$. Käsitellyn jäteveden kiintoainefraktion lääkeainepitoisuus vaihteli Keniassa välillä 11–31117 $\mu g\ kg^{-1}$. Lääkeaineiden tarttuminen jäteveden kiintoaineeseen, lietteeseen ja sedimentteihin todettiin olevan erittäin merkittävä reitti ympäristöön. Kirjallisuudessa mainitaan yleensä vain lääkeaineet suodatetuista näytteistä, jolloin saadaan aliarvioitu kuva ympäristöön päätyvistä lääkeainemääristä.

Jäteveden puhdistamoissa Keniassa ja Sambiassa ei tapahtunut juurikaan lääkeaineiden poistumista. Pitoisuudet saattoivat joskus jopa kasvaa (-322 %), koska lääkeaineita huuhtoutui karkaavan kiintoaineen mukana vastaanottajavesistöön ja metaboloituneet lääkeaineet hajosivat puhdistamossa takaisin aktiiviainemolekyyliksi. Jyväskylän käsitellyn yhdyskuntajäteveden tutkittujen aktiiviaineiden yhteismäärä oli korkeimmillaan nanogrammoja litrassa, kun taas Keniassa ja Sambiassa tasot olivat mikrogrammoja litrassa:

Tutkimuksessa tarkasteltujen jokivesistöjen lääkeaineiden yhteispitoisuudet olivat korkeita (96 µg l^{-1}) jo ennen kuin niihin johdettiin käsiteltyjä jätevesiä, jonka jälkeen liukoiset lääkeainepitoisuudet kohosivat (142 µg l^{-1}). Tämä johtuu asutuksen ja karjatalouden aiheuttamasta hajakuormituksesta. Jokivesiä käytetään sellaisenaan kastelu- ja pesuvesinä. Myös avoviemärien vettä käytetään kasteluun ainakin kuivina kausina. Jätevesiä vastaanottavien jokien sedimentin lääkeainepitoisuudet olivat purkupaikan alapuolella jopa neljä kertaa suurempia kuin ennen purkupaikkaa (4 125 µg l^{-1}). Kaikissa tutkituissa näytteissä esiintyi eniten siprofloksasiinia, sulfametoksatsolia ja trimetopriimia.

Lusakassa tutkittiin lääkeaineita myös tutkimusalueiden kaivoista. Pohjaveden lääkeainepitoisuudet vaihtelivat alle määritysrajasta tasolle mikrogrammoja litrassa: siprofloksasiini 0,14, norfoksasiini 0,14 ja amoksilliini 0,66 µg l^{-1}. Havaittujen lääkeainepitoisuuksien avulla arvioitiin laskennallista riskiä antibioottiresistenssin synnylle. Laskennallinen riski vaihteli välillä <0,1 ja 53, mikä osoittaa keskisuuren tai suuren antibioottiresistenssin kehittymisen riskin. Sambian Lusakassa ympäristömikrobiomissa ja jätevesissä havaittiin lukuisia antibioottiresistenssigeenejä, jotka antavat vastustuskyvyn sulfonamideja, trimetopriimiä, tetrasykliiniä, kinoloneja, makrolideja, rifamysiiniä ja β-laktaameja (mukaan lukien karbapeneemit) vastaan. Antibioottiresistenssigeenien esiintyvyys jätevesissä oli usean geenin kohdalla korkea, mutta pohjavedessä niitä ei havaittu.

Järjestetty jätevesien keräily ja käsittely on harvinaista kehitysmaissa, tai jos keskitetty sanitaatio onkin olemassa, antibiootit ja antibioottiresistenssigeenit poistuvat vain osittain käsittelyssä, varsinkin Keniassa ja Sambiassa. Tämän takia työssä pyrittiin vähentämään lääkeaineiden pääsyä ympäristöön tai ylikuormitettuun ja heikosti toimivaan jätevesien keräys- ja käsittelyjärjestelmään kuivasanitaation avulla. Siinä virtsa ja ulosteet kerätään erikseen ja lääkeaineita sisältävä virtsa voidaan käsitellä pienessä tilavuudessa mutta korkeassa konsentraatiossa useilla eri tekniikoilla. Tässä työssä keskityttiin lääkeaineiden poistoon aktiivihiilisuodatuksen avulla. Lääkeaineet tarttuvat aktiivihiileen, joka voidaan erottaa nestefaasista ja käsitelty virtsa voidaan hyödyntää turvallisesti lannoitteena ja kasteluvetenä.

Ajatus lääkeaineiden talteenotosta ennen kuin ne päätyvät ympäristöön tai jätevesijärjestelmään on houkutteleva, sillä erillisvirtauskeräyksessä puhutaan muutamista litroista per henkilö päivässä. Veteen perustuva sanitaatiojärjestelmä laimentaa lääkeainepitoisuudet hyvin pieniksi mutta satakertaistaa käsiteltävän nestemäärän. Kirjallisuudesta löytyy runsaasti laboratorio- ja pilot-tutkimuksia, jotka osoittavat, että hyvin erilaiset ja paikallisista ja edullisista lähtöaineita tehdyt adsorbentit pystyvät poistamaan lääkeaineet erilliskerätystä virtsasta. Erilliskerätty virtsa sisälsi aikaisempien tutkimuksiemme perusteella lääkeaineita yhteensä useita kymmeniä milligrammoja litrassa. Korkeimmillaan pitoisuudet olivat suilfametaksatsoli 7 740 µg l^{-1}, trimetopriimi 12 800 µg l^{-1} ja antiviraalinen lääkeaine

lamivudiini 10 010 µg l^{-1}. Laboratoriokokeissa lisättiin fysikaalisilta ja kemiallisilta ominaisuuksiltaan erilaisia lääkeaineita 15 mg litraan hydrolysoitua virtsaa. Puupohjainen aktiivihiilivalmiste pystyi poistamaan ravistelukokeessa lähes kaikki lääkeaineet virtsasta annoksella, joka vastaa paria ruokalusikallista pulverimaista aktiivihiiltä virtsalitraa kohti. Tällaista yksinkertaista tekniikkaa käyttäen voidaan vähentää ympäristön lääkeainekuormitusta. Käsitelty virtsa on turvallinen ravinteiden, typen, fosforin ja kaliumin lähde. Tekniikka voi toimia panosperiaatteella ravistelemalla tai käsittely voidaan tuotteistaa esimerkiksi suodatinratkaisuksi ja skaalata suurempaan mittakaavaan. Tuotekehitys virtsan keräyksen, varastoinnin ja aktiivihiilen regeneroinnin tai hyödyntämisen suhteen tulee olemaan jatkotutkimuksen aihe yhteistyössä Jomo Kenyatta University of Agriculture and Technologyn kanssa.

REFERENCES

Adeola A.O. & Forbes P.B.C. 2022. Antiretroviral Drugs in African Surface Waters: Prevalence, Analysis, and Potential Remediation. *Environmental Toxicology and Chemistry* 41: 247–262.

Adhikari S. & Halden R.U. 2022. Opportunities and limits of wastewater-based epidemiology for tracking global health and attainment of UN sustainable development goals. *Environment International* 163: 1–9.

Ågerstrand M., Josefsson H., Wernersson A.S. & Larsson D.G.J. 2023. Opportunities to tackle antibiotic resistance development in the aquatic environment through the Water Framework Directive. *Ambio* 52: 941–951.

Agunbiade F.O. & Moodley B. 2016. Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa. *Environmental Toxicology and Chemistry* 35: 36–46.

Akenga P., Gachanja A., Fitzsimons M.F., Tappin A. & Comber S. 2021. Uptake, accumulation and impact of antiretroviral and antiviral pharmaceutical compounds in lettuce. *Science of the Total Environment* 766: 144499.

Al-Khazrajy, Omar S. A. and Alistair B.A.B. 2017. Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC-DAD or LC-ESI-MS/MS detection. *Analytical Methods* 9: 4190–4200.

Andersson D.I. & Hughes D. 2014. Microbiological effects of sublethal levels of antibiotics. *Nature Reviews Microbiology* 12: 465–478.

Anjum M.F. 2015. Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. *Future Microbiology* 10: 317–320.

Bagnis S., Boxall A., Gachanja A., Fitzsimons M., Murigi M., Snape J., Tappin A., Wilkinson J. & Comber S. 2020. Characterization of the Nairobi River catchment impact zone and occurrence of pharmaceuticals: Implications for an impact zone inclusive environmental risk assessment. *Science of the Total Environment* 703: 134925.

Barbosa S.G., Rodrigues T., Peixoto L., Kuntke P., Alves M.M., Pereira M.A. & Ter Heijne A. 2019. Anaerobic biological fermentation of urine as a strategy to enhance the performance of a microbial electrolysis cell (MEC). *Renewable Energy* 139: 936–943.

Beek T. aus der, Weber F.-A., Bergmann A., Hickmann S., Ebert I., Hein A. & Küster A. 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. *Environmental Toxicology and Chemistry* 35: 823–835.

Bengtsson-Palme J. & Larsson D.G.J. 2015. Antibiotic resistance genes in the environment: prioritizing risks. *Nature Reviews Microbiology* 13: 396–396.

Bengtsson-Palme J. & Larsson D.G.J. 2016. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environment International* 86: 140–149.

Bengtsson-Palme J., Milakovic M., Švecová H., Ganjto M., Jonsson V., Grabic R. & Udikovic-Kolic N. 2019. Industrial wastewater treatment plant enriches antibiotic resistance genes and alters the structure of microbial communities. *Water Research* 162: 437–445.

Bischel H.N., Özel Duygan B.D., Strande L., McArdell C.S., Udert K.M. & Kohn T. 2015. Pathogens and pharmaceuticals in source-separated urine in eThekwini, South Africa. *Water Research* 85: 57–65.

Blakely G.W. 2015. Mechanisms of Horizontal Gene Transfer and DNA Recombination. *Molecular Medical Microbiology* 1: 291–302.

Boulard L., Dierkes G., Schlüsener M.P., Wick A., Koschorreck J. & Ternes T.A. 2020. Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers. *Water Research* 171: 115366.

Boxall A.B.A. 2004. The environmental side effects of medication. *EMBO reports* 5: 1110–1116.

Brinkac L., Voorhies A., Gomez A. & Nelson K.E. 2017. The Threat of Antimicrobial Resistance on the Human Microbiome. *Microbial Ecology* 74: 1001–1008.

Brito I.L. 2021. Examining horizontal gene transfer in microbial communities. *Nature Reviews Microbiology* 19: 442–453.

Brown A.K., Ackerman J., Cicek N. & Wong C.S. 2020. In situ kinetics of human pharmaceutical conjugates and the impact of transformation, deconjugation, and sorption on persistence in wastewater batch bioreactors. *Environmental Pollution* 265: 114852.

Bürgmann H., Frigon D., Gaze W.H., Manaia C.M., Pruden A., Singer A.C., Smets B.F. & Zhang T. 2018. Water and sanitation: An essential battlefront in the war on antimicrobial resistance. *FEMS Microbiology Ecology* 94: 1–14.

Cantoni B., Ianes J., Bertolo B., Ziccardi S., Maffini F. & Antonelli M. 2024. Adsorption on activated carbon combined with ozonation for the removal of contaminants of emerging concern in drinking water. *Journal of Environmental Management* 350: 119537.

Castaño-Trias M., Rodríguez-Mozaz S. & Buttiglieri G. 2023. A decade of water monitoring in a Mediterranean region: Pharmaceutical prioritisation for an upgraded analytical methodology. *Environmental Nanotechnology, Monitoring & Management* 20: 100850.

Chauhan S., Shafi T., Brajesh ·, Dubey K. & Chowdhury S. 2023. Biochar-mediated removal of pharmaceutical compounds from aqueous matrices via adsorption. 5: 37–62.

Chen J., Wei X.D., Liu Y.S., Ying G.G., Liu S.S., He L.Y., Su H.C., Hu L.X., Chen F.R. & Yang Y.Q. 2016a. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Optimization of wetland substrates and hydraulic loading. *Science of the Total Environment* 565: 240–248.

Chen J., Ying G.G., Wei X.D., Liu Y.S., Liu S.S., Hu L.X., He L.Y., Chen Z.F., Chen F.R. & Yang Y.Q. 2016b. Removal of antibiotics and antibiotic resistance genes

from domestic sewage by constructed wetlands: Effect of flow configuration and plant species. *Science of the Total Environment* 571: 974–982.

Cheng N., Wang B., Wu P., Lee X., Xing Y., Chen M. & Gao B. 2021. Adsorption of emerging contaminants from water and wastewater by modified biochar: A review. *Environmental Pollution* 273: 116448.

Collignon P., Beggs J.J., Walsh T.R., Gandra S. & Laxminarayan R. 2018. Articles Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *The Lancet Planet Health* 2: e398-405.

Cook J.D., Strauss K.A., Caplan Y.H., Todico C.P. & Bush D.M. 2007. Urine pH: the Effects of Time and Temperature after Collection. *Journal of Analytical Toxicology* 31: 486–496.

Costa F., Lago A., Rocha nica, Barros scar, Costa L., Vipotnik Z., Silva B. & Tavares T. 2019. A Review on Biological Processes for Pharmaceuticals Wastes Abatement: A Growing Threat to Modern Society. *Environ. Sci. Technol.* 53: 7185–7202.

Dadgostar P. 2019. Antimicrobial resistance: implications and costs. *Infection and Drug Resistance* 12: 3903–3910.

Demissie N., Simha P., Lai F.Y., Ahrens L., Mussabek D., Desta A. & Vinnerås B. 2023. Degradation of 75 organic micropollutants in fresh human urine and water by UV advanced oxidation process. *Water Research* 242: 120221.

Deshpande A.S. & Fahrenfeld N.L. 2022. Abundance, diversity, and host assignment of total, intracellular, and extracellular antibiotic resistance genes in riverbed sediments. *Water Research* 217: 118363.

Duygan B.D.Ö., Udert K.M., Remmele A. & McArdell C.S. 2021. Removal of pharmaceuticals from human urine during storage, aerobic biological treatment, and activated carbon adsorption to produce a safe fertilizer. *Resources, Conservation and Recycling* 166: 105341.

Ebmeyer S., Kristiansson E. & Larsson D.G.J. 2021. A framework for identifying the recent origins of mobile antibiotic resistance genes. *Communications Biology* 4: 1–10.

EC. 2017. *A European one health action plan against antimicrobial resistance*. European commission, Brussels, Belgium.

Emamalipour M., Seidi K., Zununi Vahed S., Jahanban-Esfahlan A., Jaymand M., Majdi H., Amoozgar Z., Chitkushev L.T., Javaheri T., Jahanban-Esfahlan R. & Zare P. 2020. Horizontal Gene Transfer: From Evolutionary Flexibility to Disease Progression. *Frontiers in Cell and Developmental Biology* 8: 229.

Emara Y., Jolliet O., Finkbeiner M., Heß S., Kosnik M., Siegert M.-W. & Fantke P. 2023. Comparative selective pressure potential of antibiotics in the environment. *Environmental Pollution* 318: 120873.

EurEau. 2019. *Study on the Feasibility of Applying Extended Producer Responsibility to Micropollutants And Microplastics Emitted in the Aquatic Environment from Products During Their Life Cycle*. EurEau, Bruselles, Belgium.

Fahad A., Saphira Mohamed R.M., Radhi B. & Al-Sahari M. 2019. Wastewater and its Treatment Techniques: An Ample Review. *Indian Journal of Science and Technology* 12: 1–13.

Fan-Havard P., Liu Z., Chou M., Ling Y., Barrail-Tran A., Haas D.W., Taburet A.-M. & ANRS12154 Study Group the A.S. 2013. Pharmacokinetics of phase I nevirapine metabolites following a single dose and at steady state. *Antimicrobial agents and chemotherapy* 57: 2154–2160.

Fatta-Kassinos D., Meric S. & Nikolaou A. 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and Bioanalytical Chemistry* 399: 251–275.

Galhano B.S.P., Ferrari R.G., Panzenhagen P., Jesus A.C.S. de & Conte-Junior C.A. 2021. Antimicrobial resistance gene detection methods for bacteria in animal-based foods: A brief review of highlights and advantages. *Microorganisms* 9: 1–15.

Gautam A. 2022. Antimicrobial Resistance: The Next Probable Pandemic. *Journal of the Nepal Medical Association* 60: 225–228.

Gelband H., Molly Miller P., Pant S., Gandra S., Levinson J., Barter D., White A. & Laxminarayan R. 2015. The state of the world's antibiotics 2015. *Wound Healing Southern Africa* 8: 30–34.

Gildemeister D., Moermond C.T.A., Berg C., Bergstrom U., Bielská L., Evandri M.G., Franceschin M., Kolar B., Montforts M.H.M.M. & Vaculik C. 2023. Improving the regulatory environmental risk assessment of human pharmaceuticals: Required changes in the new legislation. *Regulatory Toxicology and Pharmacology* 142: 105437.

Graham D.W., Bergeron G., Bourassa M.W., Dickson J., Gomes F., Howe A., Kahn L.H., Morley P.S., Scott H.M., Simjee S., Singer R.S., Smith T.C., Storrs C. & Wittum T.E. 2019. Complexities in understanding antimicrobial resistance across domesticated animal, human, and environmental systems. *Annals of the New York Academy of Sciences* 1441: 17–30.

Gullberg E., Cao S., Berg O.G., Ilbäck C., Sandegren L., Hughes D., Andersson D.I. & Lipsitch M. 2011. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *PLoS Pathogens* 7: e1002158.

Gupta S., Mittal Y., Panja R. & Prajapati K.B. 2021. Conventional wastewater treatment technologies. In: *Current Developments in Biotechnology and Bioengineering: Strategic Perspectives in Solid Waste and Wastewater Management*, Elsevier, Amsterdam, Netherlands, pp. 47–75.

Häfner F., Monzon Diaz O.R., Tietjen S., Schröder C. & Krause A. 2023. Recycling fertilizers from human excreta exhibit high nitrogen fertilizer value and result in low uptake of pharmaceutical compounds. *Frontiers in Environmental Science* 10: 1038175.

Hall J.P.J., Brockhurst M.A. & Harrison E. 2017. Sampling the mobile gene pool: Innovation via horizontal gene transfer in bacteria. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372: 1–10.

Hanna N., Sun P., Sun Q., Li X., Yang X., Ji X., Zou H., Ottoson J., Nilsson L.E., Berglund B., Dyar O.J., Tamhankar A.J. & Stålsby Lundborg C. 2018. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: Its potential for resistance development and ecological and human risk. *Environment International* 114: 131–142.

Heinonen-Tanski H., Pradhan S.K. & Karinen P. 2010. Sustainable sanitation-A cost-effective tool to improve plant yields and the environment. *Sustainability* 2: 341–353.

Heinonen-Tanski H. & Van Wijk-Sijbesma C. 2005. Human excreta for plant production. *Bioresource Technology* 96: 403–411.

Hendriksen R.S., Munk P., Njage P., Bunnik B. van, McNally L., Lukjancenko O., Röder T., Nieuwenhuijse D., Pedersen S.K., Kjeldgaard J., Kaas R.S., Clausen P.T.L.C., Vogt J.K., Leekitcharoenphon P., Schans M.G.M. van de, Zuidema T., Roda Husman A.M. de, Rasmussen S., Petersen B., Bego A., Rees C., Cassar S., Coventry K., Collignon P., Allerberger F., Rahube T.O., Oliveira G., Ivanov I., Vuthy Y., Sopheak T., Yost C.K., Ke C., Zheng H., Baisheng L., Jiao X., Donado-Godoy P., Coulibaly K.J., Jergović M., Hrenovic J., Karpíšková R., Villacis J.E., Legesse M., Eguale T., Heikinheimo A., Malania L., Nitsche A., Brinkmann A., Saba C.K.S., Kocsis B., Solymosi N., Thorsteinsdottir T.R., Hatha A.M., Alebouyeh M., Morris D., Cormican M., O'Connor L., Moran-Gilad J., Alba P., Battisti A., Shakenova Z., Kiiyukia C., Ng'eno E., Raka L., Avsejenko J., Bērziņš A., Bartkevics V., Penny C., Rajandas H., Parimannan S., Haber M.V., Pal P., Jeunen G.J., Gemmell N., Fashae K., Holmstad R., Hasan R., Shakoor S., Rojas M.L.Z., Wasyl D., Bosevska G., Kochubovski M., Radu C., Gassama A., Radosavljevic V., Wuertz S., Zuniga-Montanez R., Tay M.Y.F., Gavačová D., Pastuchova K., Truska P., Trkov M., Esterhuyse K., Keddy K., Cerdà-Cuéllar M., Pathirage S., Norrgren L., Örn S., Larsson D.G.J., et al. 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nature Communications* 10: 1124.

Hunter P.R., Wilkinson D.C., Catling L.A. & Barker G.C. 2008. Meta-Analysis of Experimental Data Concerning Antimicrobial Resistance Gene Transfer Rates during Conjugation. *Applied and Environmental Microbiology* 74: 6085–6090.

Imwene K.O., Ngumba E. & Kairigo P.K. 2022. Emerging technologies for enhanced removal of residual antibiotics from source-separated urine and wastewaters: A review. *Journal of Environmental Management* 322: 116065.

Inyinbor A.A., Bello O.S., Fadiji A.E. & Inyinbor H.E. 2018. Threats from antibiotics: A serious environmental concern. *Journal of Environmental Chemical Engineering* 6: 784–793.

Ishii S.K.L. & Boyer T.H. 2015. Life cycle comparison of centralized wastewater treatment and urine source separation with struvite precipitation: Focus on urine nutrient management. *Water Research* 79: 88–103.

Jaatinen S.T., Palmroth M.R.T., Rintala J.A. & Tuhkanen T.A. 2016. The effect of urine storage on antiviral and antibiotic compounds in the liquid phase of source-separated urine. *Environmental Technology* 37: 2189–2198.

Jelic A., Rodriguez-Mozaz S., Barceló D. & Gutierrez O. 2015. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Research* 68: 98–108.

Jermakka J., Freguia S., Kokko M. & Ledezma P. 2021. Electrochemical system for selective oxidation of organics over ammonia in urine. *Environ. Sci. Water Res. Technol* 7: 942.

Jiao J., Lv X., Shen C. & Morigen M. 2024. Genome and transcriptomic analysis of the adaptation of *Escherichia coli* to environmental stresses. *Computational and Structural Biotechnology Journal* 23: 2132–2140.

Jjemba P.K. 2006. Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. *Ecotoxicology and Environmental Safety* 63: 113–130.

Joss A., Keller E., Alder A.C., Göbel A., McArdell C.S., Ternes T. & Siegrist H. 2005. Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Research* 39: 3139–3152.

Juhas M., Meer J.R. van der, Gaillard M., Harding R.M., Hood D.W. & Crook D.W. 2009. Genomic islands: tools of bacterial horizontal gene transfer and evolution. *FEMS Microbiology Reviews* 33: 376–393.

Kaeseberg T., Zhang J., Schubert S., Oertel R. & Krebs P. 2018. Abiotic, biotic and photolytic degradation affinity of 14 antibiotics and one metabolite – batch experiments and a model framework. 241: 339–350.

Karkman A., Do T.T., Walsh F. & Virta M.P.J. 2018. Antibiotic-Resistance Genes in Waste Water. *Trends in Microbiology* 26: 220–228.

Kasprzyk-Hordern B., Dinsdale R.M. & Guwy A.J. 2007. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A* 1161: 132–145.

Kasprzyk-Hordern B., Proctor K., Jagadeesan K., Watkins S., Standerwick R., Barden R. & Barnett J. 2021. Diagnosing Down-the-Drain Disposal of Unused Pharmaceuticals at a River Catchment Level: Unrecognized Sources of Environmental Contamination That Require Nontechnological Solutions. *Environmental Science and Technology* 55: 11657–11666.

Kelova M.E., Eich-Greatorex S. & Krogstad T. 2021. Human excreta as a resource in agriculture – Evaluating the fertilizer potential of different composting and fermentation-derived products. *Resources, Conservation and Recycling* 175: 105748.

Khasawneh O.F.S. & Palaniandy P. 2021. Occurrence and removal of pharmaceuticals in wastewater treatment plants. *Process Safety and Environmental Protection* 150: 532–556.

Kim D.W. & Cha C.J. 2021. Antibiotic resistome from the One-Health perspective: understanding and controlling antimicrobial resistance transmission. *Experimental and Molecular Medicine* 53: 301–309.

Kim S., Yun Z., Ha U.H., Lee S., Park H., Kwon E.E., Cho Y., Choung S., Oh J., Medriano C.A. & Chandran K. 2014. Transfer of antibiotic resistance plasmids in pure and activated sludge cultures in the presence of environmentally representative micro-contaminant concentrations. *Science of the Total Environment* 468–469: 813–820.

Kirchhelle C. 2023. The Antibiocene – towards an eco-social analysis of humanity's antimicrobial footprint. *Humanities and Social Sciences Communications* 10: 619.

Klein E.Y., Van Boeckel T.P., Martinez E.M., Pant S., Gandra S., Levin S.A., Goossens H. & Laxminarayan R. 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *PNAS* 115: E3463–E3470.

Köpping I., McArdell C.S., Borowska E., Böhler M.A. & Udert K.M. 2020. Removal of pharmaceuticals from nitrified urine by adsorption on granular activated carbon. *Water Research X* 9: 100057.

K'oreje K.O., Okoth M., Van Langenhove H. & Demeestere K. 2020. Occurrence and treatment of contaminants of emerging concern in the African aquatic environment: Literature review and a look ahead. *Journal of Environmental Management* 254: 109752.

K'oreje K.O., Vergeynst L., Ombaka D., De Wispelaere P., Okoth M., Van Langenhove H. & Demeestere K. 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere* 149: 238–244.

Kotwani A., Joshi J. & Kaloni D. 2021. Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance. *Environmental Science and Pollution Research* 28: 32111–32124.

Kovalova L., Siegrist H., Von Gunten U., Eugster J., Hagenbuch M., Wittmer A., Moser R. & McArdell C.S. 2013. Elimination of micropollutants during post-treatment of hospital wastewater with powdered activated carbon, ozone, and UV. *Environmental Science and Technology* 47: 7899–7908.

Kümmerer K. 2003. Significance of antibiotics in the environment. *Journal of Antimicrobial Chemotherapy* 52: 5–7.

Kümmerer K. 2008. *Pharmaceuticals in the Environment*. Springer, Berlin, Heidelberg.

Kümmerer K. 2009a. Antibiotics in the aquatic environment – A review – Part II. *Chemosphere* 75: 435–441.

Kümmerer K. 2009b. The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. *Journal of Environmental Management* 90: 2354–2366.

Kuntke P., Rodrigues M., Sleutels T., Saakes M., Hamelers H.V.M. & Buisman C.J.N. 2018. Energy-Efficient Ammonia Recovery in an Up-Scaled Hydrogen Gas

Recycling Electrochemical System. *ACS Sustainable Chemistry and Engineering* 6: 7638–7644.

Kusi J., Ojewole C.O., Ojewole A.E. & Nwi-Mozu I. 2022. Antimicrobial Resistance Development Pathways in Surface Waters and Public Health Implications. *Antibiotics* 11: 821.

Larsen T.A., Gruendl H. & Binz C. 2021a. The potential contribution of urine source separation to the SDG agenda – A review of the progress so far and future development options†. *Environmental Science: Water Research and Technology* 7: 1161–1176.

Larsen T.A., Maurer M., Udert K.M. & Lienert J. 2007. Nutrient cycles and resource management: Implications for the choice of wastewater treatment technology. *Water Science and Technology* 56: 229–237.

Larsen T.A., Riechmann M.E. & Udert K.M. 2021b. State of the art of urine treatment technologies: A critical review. *Water Research X* 13: 100114.

Larsson D.G.J., Andremont A., Bengtsson-Palme J., Brandt K.K., Roda Husman A.M. de, Fagerstedt P., Fick J., Flach C.-F., Gaze W.H., Kuroda M., Kvint K., Laxminarayan R., Manaia C.M., Nielsen K.M., Plant L., Ploy M.-C., Segovia C., Simonet P., Smalla K., Snape J., Topp E., Hengel A.J. van, Verner-Jeffreys D.W., Virta M.P.J., Wellington E.M. & Wernersson A.-S. 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. *Environment International* 117: 132–138.

Larsson D.G.J. & Flach C.F. 2021. Antibiotic resistance in the environment. *Nature Reviews Microbiology* 20: 257–269.

Ledieu L., Simonneau A., Thiebault T., Fougere L., Destandau E., Cerdan O. & Laggoun F. 2021. Spatial distribution of pharmaceuticals within the particulate phases of a peri-urban stream. *Chemosphere* 279: 130385.

Li Y., Niu X., Yao C., Yang W. & Lu G. 2019. Distribution, removal, and risk assessment of pharmaceuticals and their metabolites in five sewage plants. *International Journal of Environmental Research and Public Health* 16: 4729.

Li Z., Su M., Cheng W., Xia J., Liu S., Liu R., Sun S., Feng L., Zhu X., Zhang X., Tian X. & Qu L. 2021. Pharmacokinetics, Urinary Excretion, and Pharmaco-Metabolomic Study of Tebipenem Pivoxil Granules After Single Escalating Oral Dose in Healthy Chinese Volunteers. *Frontiers in Pharmacology* 12: 696165.

Li D., Zeng S., He M. & Gu A.Z. 2016. Water Disinfection Byproducts Induce Antibiotic Resistance-Role of Environmental Pollutants in Resistance Phenomena. *Environmental science & technology* 50: 3193–3201.

Lindberg R.H., Olofsson U., Rendahl P., Johansson M.I., Tysklind M. & Andersson B.A.V. 2006. Behavior of fluoroquinolones and trimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. *Environmental Science and Technology* 40: 1042–1048.

Lindberg R.H., Wennberg P., Johansson M.I., Tysklind M. & Andersson B.A.V. 2005. Screening of human antibiotic substances and determination of weekly mass

flows in five sewage treatment plants in Sweden. *Environmental Science and Technology* 39: 3421–3429.

Liu A., Fong A., Becket E., Yuan J., Tamae C., Medrano L., Maiz M., Wahba C., Lee C., Lee K., Tran K.P., Yang H., Hoffman R.M., Salih A. & Miller J.H. 2011. Selective Advantage of Resistant Strains at Trace Levels of Antibiotics: a Simple and Ultrasensitive Color Test for Detection of Antibiotics and Genotoxic Agents. *Antimicrobial Agents and Chemotherapy* 55: 1204–1210.

Liu M., Hata A., Katayama H. & Kasuga I. 2020. Consecutive ultrafiltration and silica adsorption for recovery of extracellular antibiotic resistance genes from an urban river. *Environmental Pollution* 260: 114062.

Liu Y., He L.F., Deng Y.Y., Zhang Q., Jiang G.M. & Liu H. 2022. Recent progress on the recovery of valuable resources from source-separated urine on-site using electrochemical technologies: A review. *Chemical Engineering Journal* 442: 136200.

López-Serna R., Jurado A., Vázquez-Suñé E., Carrera J., Petrović M. & Barceló D. 2013. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. *Environmental Pollution* 174: 305–315.

Lu C., Wang J., Pan L., Gu X., Lu W., Chen D., Zhang C., Ye Q., Xiao C., Liu P., Tang Y., Tang B., Huang G., Fang J. & Jiang H. 2023. Rapid detection of multiple resistance genes to last-resort antibiotics in Enterobacteriaceae pathogens by recombinase polymerase amplification combined with lateral flow dipstick. *Frontiers in Microbiology* 13: 1062577.

Maddela N.R., García L.C. & Chakraborty C.S. (eds.). 2021. *Environmental and Microbial Biotechnology Advances in the Domain of Environmental Biotechnology Microbiological Developments in Industries, Wastewater Treatment and Agriculture*. Springer, Singapore.

Mahaye N. & Musee N. 2022. Effects of Two Antiretroviral Drugs on the Crustacean Daphnia magna in River Water. *Toxics* 10: 423.

Malmqvist E., Fumagalli D., Munthe C. & Larsson D.G.J. 2023. Pharmaceutical Pollution from Human Use and the Polluter Pays Principle. *Public Health Ethics* 16: 152–164.

Mashiane M S.C. 2015. Quantification of Selected Antiretroviral Drugs in a Wastewater Treatment Works in South Africa Using GC-TOFMS. *Journal of Chromatography & Separation Techniques* 06: 272.

Matongo S., Birungi G., Moodley B. & Ndungu P. 2015a. Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. *Environmental Science and Pollution Research* 22: 10298–10308.

Matongo S., Birungi G., Moodley B. & Ndungu P. 2015b. Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. *Chemosphere* 134: 133–140.

Maurer M., Pronk W. & Larsen T.A. 2006. Treatment processes for source-separated urine. *Water Research* 40: 3151–3166.

McEachran A.D., Shea D., Bodnar W. & Nichols E.G. 2016. Pharmaceutical occurrence in groundwater and surface waters in forests land-applied with municipal wastewater. *Environmental Toxicology and Chemistry* 35: 898–905.

Mcinnes R.S., Hassan Uz-Zaman M., Alam I.T., Fung S., Ho S., Moran R.A., Clemens J.D., Islam S. & Van Schaik W. 2021. Metagenome-Wide Analysis of Rural and Urban Surface Waters and Sediments in Bangladesh Identifies Human Waste as a Driver of Antibiotic Resistance. 6: e00137-21.

Michael I., Rizzo L., McArdell C.S., Manaia C.M., Merlin C., Schwartz T., Dagot C. & Fatta-Kassinos D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research* 47: 957–995.

Miller L.D., Russell M.H. & Alexandre G. 2009. Chapter 3 Diversity in Bacterial Chemotactic Responses and Niche Adaptation. In: *Advances in Applied Microbiology*, Academic Press, pp. 53–75.

Moermond C.T.A., Berg C., Bergstrom U., Bielská L., Evandri M.G., Franceschin M., Gildemeister D. & Montforts M.H.M.M. 2023. Proposal for regulatory risk mitigation measures for human pharmaceutical residues in the environment. *Regulatory Toxicology and Pharmacology* 143: 105443.

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

Munita J.M. & Arias C.A. 2016. Mechanisms of Antibiotic Resistance. *Microbiology Spectrum* 4: 1–24.

Murray L.M., Hayes A., Snape J., Kasprzyk-Hordern B., Gaze W.H. & Murray A.K. 2024. Co-selection for antibiotic resistance by environmental contaminants. *npj Antimicrobials and Resistance* 2: 9.

Murray C.J., Ikuta K.S., Sharara F., Swetschinski L., Robles Aguilar G., Gray A., Han C., Bisignano C., Rao P., Wool E., Johnson S.C., Browne A.J., Chipeta M.G., Fell F., Hackett S., Haines-Woodhouse G., Kashef Hamadani B.H., Kumaran E.A.P., McManigal B., Agarwal R., Akech S., Albertson S., Amuasi J., Andrews J., Aravkin A., Ashley E., Bailey F., Baker S., Basnyat B., Bekker A., Bender R., Bethou A., Bielicki J., Boonkasidecha S., Bukosia J., Carvalheiro C., Castañeda-Orjuela C., Chansamouth V., Chaurasia S., Chiurchiù S., Chowdhury F., Cook A.J., Cooper B., Cressey T.R., Criollo-Mora E., Cunningham M., Darboe S., Day N.P.J., De Luca M., Dokova K., Dramowski A., Dunachie S.J., Eckmanns T., Eibach D., Emami A., Feasey N., Fisher-Pearson N., Forrest K., Garrett D., Gastmeier P., Giref A.Z., Greer R.C., Gupta V., Haller S., Haselbeck A., Hay S.I., Holm M., Hopkins S., Iregbu K.C., Jacobs J., Jarovsky D., Javanmardi F., Khorana M., Kissoon N., Kobeissi E., Kostyanev T., Krapp F., Krumkamp R., Kumar A., Kyu H.H., Lim C., Limmathurotsakul D., Loftus M.J., Lunn M., Ma J., Mturi N., Munera-Huertas T., Musicha P., Mussi-Pinhata M.M., Nakamura T., Nanavati R., Nangia S., Newton P., Ngoun C., Novotney A., Nwakanma

D., Obiero C.W., Olivas-Martinez A., et al. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399: 629–655.

Nadimpalli M.L., Marks S.J., Montealegre M.C., Gilman R.H., Pajuelo M.J., Saito M., Tsukayama P., Njenga S.M., Kiiru J., Swarthout J., Islam M.A., Julian T.R. & Pickering A.J. 2020. Urban informal settlements as hotspots of antimicrobial resistance and the need to curb environmental transmission. *Nature Microbiology* 5: 787–795.

Nannou C., Ofrydopoulou A., Evgenidou E., Heath D., Heath E. & Lambropoulou D. 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. *Science of the Total Environment* 699: 134322.

Nantaba F., Wasswa J., Kylin H., Palm W.U., Bouwman H. & Kümmerer K. 2020. Occurrence, distribution, and ecotoxicological risk assessment of selected pharmaceutical compounds in water from Lake Victoria, Uganda. *Chemosphere* 239: 124642.

Ncube S., Madikizela L.M., Chimuka L. & Nindi M.M. 2018. Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. *Water Research* 145: 231–247.

Ngumba E. 2020. Removal of selected antibiotics and antiretroviral drugs during post-treatment of municipal wastewater with UV , UV / chlorine and UV / hydrogen peroxide. *Water and Environment Journal* 1: 1–12.

Ngumba E., Gachanja A., Nyirenda J., Maldonado J. & Tuhkanen T. 2020. Occurrence of antibiotics and antiretroviral drugs in source-separated urine, groundwater, surface water and wastewater in the peri-urban area of chunga in lusaka, Zambia. *Water SA* 46: 278–284.

Ngumba E., Päivi K., Gachanja A. & Tuhkanen T. 2016a. A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards. *Anal meth* 8: 6720.

Ngumba E., Gachanja A. & Tuhkanen T. 2016b. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of The Total Environment* 539: 206–213.

Nguyen P.Y., Carvalho G., Polesel F., Torresi E., Rodrigues A.M., Rodrigues J.E., Cardoso V.V., Benoliel M.J., Plósz B.G., Reis M.A.M. & Oehmen A. 2018. Bioaugmentation of activated sludge with Achromobacter denitrificans PR1 for enhancing the biotransformation of sulfamethoxazole and its human conjugates in real wastewater: Kinetic tests and modelling. *Chemical Engineering Journal* 352: 79–89.

Obaideen K., Shehata N., Sayed E.T., Abdelkareem M.A., Mahmoud M.S. & Olabi A.G. 2022. The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. *Energy Nexus* 7: 100112.

Öberg G., Metson G.S., Kuwayama Y. & Conrad S.A. 2020. Conventional sewer systems are too time-consuming, costly and inflexible to meet the challenges of the 21st century. *Sustainability (Switzerland)* 12: 6518.

Ohoro C.R., Adeniji A.O., Elsheikh E.A.E., Al-Marzouqi A., Otim M., Okoh O.O. & Okoh A.I. 2022. Influence of physicochemical parameters on PPCP occurrences in the wetlands. *Environmental Monitoring and Assessment* 194: 339.

Onu M.A., Ayeleru O.O., Oboirien B. & Olubambi P.A. 2023. Challenges of wastewater generation and management in sub-Saharan Africa: A Review. *Environmental Challenges* 11: 100686.

Otieno A.O., Home P.G., Raude J.M., Murunga S.I., Ngumba E., Ojwang D.O. & Tuhkanen T. 2021. Pineapple peel biochar and lateritic soil as adsorbents for recovery of ammonium nitrogen from human urine. *Journal of Environmental Management* 293: 112794.

Oyediran O.O., Ayandiran E.O., Olatubi M.I. & Olabode O. 2019. Awareness of risks associated with Self-medication among Patients attending General Out-patient Department of a Tertiary Hospital in South Western Nigeria. *International Journal of Africa Nursing Sciences* 10: 110–115.

Pan M. & Chu L.M. 2017. Fate of antibiotics in soil and their uptake by edible crops. *Science of The Total Environment* 599–600: 500–512.

Patel M., Kumar R., Kishor K., Mlsna T., Pittman C.U. & Mohan D. 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chemical Reviews* 119: 3510–3673.

Peterson E. & Kaur P. 2018. Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in Microbiology* 9: 2928.

Petrovic M., De Alda M.J.L., Diaz-Cruz S., Postigo C., Radjenovic J., Gros M. & Barcelo D. 2009. Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 367: 3979–4003.

Pistocchi A., Alygizakis N.A., Brack W., Boxall A., Cousins I.T., Drewes J.E., Finckh S., Gallé T., Launay M.A., McLachlan M.S., Petrovic M., Schulze T., Slobodnik J., Ternes T., Van Wezel A., Verlicchi P. & Whalley C. 2022a. European scale assessment of the potential of ozonation and activated carbon treatment to reduce micropollutant emissions with wastewater. *Science of the Total Environment* 848: 157124.

Pistocchi A., Andersen H.R., Bertanza G., Brander A., Choubert J.M., Cimbritz M., Drewes J.E., Koehler C., Krampe J., Launay M., Nielsen P.H., Obermaier N., Stanev S. & Thornberg D. 2022b. Treatment of micropollutants in wastewater: Balancing effectiveness, costs and implications. *Science of the Total Environment* 850: 157593.

Polesel F., Andersen H.R., Trapp S. & Plósz B.G. 2016. Removal of Antibiotics in Biological Wastewater Treatment Systems - A Critical Assessment Using the

Activated Sludge Modeling Framework for Xenobiotics (ASM-X). *Environmental Science and Technology* 50: 10316–10334.
Pozzebon E.A. & Seifert L. 2023. Emerging environmental health risks associated with the land application of biosolids: a scoping review. *Environmental Health: A Global Access Science Source* 22: 57.
Prasse C., Schlüsener M.P., Schulz R. & Ternes T. a. 2010. Antiviral drugs in wastewater and surface waters: A new pharmaceutical class of environmental relevance? *Environmental Science and Technology* 44: 1728–1735.
Prestinaci F., Pezzotti P. & Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health* 109: 309–318.
Radke M., Lauwigi C., Heinkele G., Múrdter T.E. & Letzel M. 2009. Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. *Environmental Science and Technology* 43: 3135–3141.
Reardon S. 2017. Resistance to last-ditch antibiotic has spread farther than anticipated. *Nature* 1: 1–3.
Reygaert W.C. 2018. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology* 4: 482–501.
Rizzo L., Malato S., Antakyali D., Beretsou V.G., Đolić M.B., Gernjak W., Heath E., Ivancev-Tumbas I., Karaolia P., Lado Ribeiro A.R., Mascolo G., McArdell C.S., Schaar H., Silva A.M.T. & Fatta-Kassinos D. 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Science of the Total Environment* 655: 986–1008.
Rodriguez-Valera F., Martin-Cuadrado A.-B., Ló M., Vasquez M. & Hallam S. 2016. Flexible genomic islands as drivers of genome evolution. 31: 154–160.
Rogowska J. & Zimmermann A. 2022. Household Pharmaceutical Waste Disposal as a Global Problem—A Review. *International Journal of Environmental Research and Public Health* 19: 15798.
Rossmann J., Schubert S., Gurke R., Oertel R. & Kirch W. 2014. Simultaneous determination of most prescribed antibiotics in multiple urban wastewater by SPE-LC-MS/MS. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 969: 162–170.
Rumky J., Kruglova A. & Repo E. 2022. Fate of antibiotic resistance genes (ARGs) in wastewater treatment plant: Preliminary study on identification before and after ultrasonication. *Environmental Research* 215: 114281.
Sabri N.A., Schmitt H., Van der Zaan B., Gerritsen H.W., Zuidema T., Rijnaarts H.H.M. & Langenhoff A.A.M. 2018. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. *Journal of Environmental Chemical Engineering* 8: 102245.
Saleh I.A., Zouari N. & Al-Ghouti M.A. 2020. Removal of pesticides from water and wastewater: Chemical, physical and biological treatment approaches. *Environmental Technology & Innovation* 19: 101026.
Sambaza S.S. & Naicker N. 2023. Contribution of wastewater to antimicrobial resistance: A review article. *Journal of Global Antimicrobial Resistance* 34: 23–29.

Samreen, Ahmad I., Malak H.A. & Abulreesh H.H. 2021. Environmental antimicrobial resistance and its drivers: a potential threat to public health. *Journal of Global Antimicrobial Resistance* 27: 101–111.

Schaider L.A., Rudel R.A., Ackerman J.M., Dunagan S.C. & Brody J.G. 2014. Pharmaceuticals, perfluorosurfactants, and other organic wastewater compounds in public drinking water wells in a shallow sand and gravel aquifer. *Science of the Total Environment* 468–469: 384–393.

Segura P.A., Takada H., Correa J.A., El Saadi K., Koike T., Onwona-Agyeman S., Ofosu-Anim J., Sabi E.B., Wasonga O.V., Mghalu J.M., Santos A.M. dos, Newman B., Weerts S. & Yargeau V. 2015. Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries. *Environment International* 80: 89–97.

Serrano M.J., García-Gonzalo D., Abilleira E., Elorduy J., Mitjana O., Falceto M.V., Laborda A., Bonastre C., Mata L., Condón S. & Pagán R. 2021. Antibacterial residue excretion via urine as an indicator for therapeutical treatment choice and farm waste treatment. *Antibiotics* 10: 762.

Sharma B., Sarkar A., Singh P. & Singh R.P. 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Management* 64: 117–132.

Simha P. & Ganesapillai M. 2017. Ecological Sanitation and nutrient recovery from human urine: How far have we come? A review. *Sustainable Environment Research* 27: 107–116.

Singer A.C., Shaw H., Rhodes V. & Hart A. 2016. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology* 7: 1–22.

Sobsey M.D., Abebe L., Andremont A., Ashbolt N.J., Husman A.M. de R., Gin K.Y.-H., Hunter P.R., Meschke J.S. & Vilchez S. 2014. Briefing Note Antimicrobial Resistance : An Emerging Water , Sanitation and Hygiene Issue. *World Health Organisation* 1: 1–16.

Solanki A. & Boyer T.H. 2017. Pharmaceutical removal in synthetic human urine using biochar. *Environmental Science Water Research & Technology* 3: 553–565.

Steffen W., Richardson K., Rockström J., Cornell S.E., Fetzer I., Bennett E.M., Biggs R., Carpenter S.R., De Vries W., De Wit C.A., Folke C., Gerten D., Heinke J., Mace G.M., Persson L.M., Ramanathan V., Reyers B. & Sörlin S. 2015. Planetary boundaries: Guiding human development on a changing planet. *Science* 347: 6223.

Subedi B., Balakrishna K., Joshua D.I. & Kannan K. 2017. Mass loading and removal of pharmaceuticals and personal care products including psychoactives, antihypertensives, and antibiotics in two sewage treatment plants in southern India. *Chemosphere* 167: 429–437.

Sun P., Li Y., Meng T., Zhang R., Song M. & Ren J. 2018. Removal of sulfonamide antibiotics and human metabolite by biochar and biochar/H2O2 in synthetic urine. *Water Research* 147: 91–100.

Tamtam F., Mercier F., Le Bot B., Eurin J., Dinh Q.T., Clément M. & Chevreuil M. 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. *Science of The Total Environment* 393: 84–95.

Tong L., Huang S., Wang Y., Liu H. & Li M. 2014. Occurrence of antibiotics in the aquatic environment of Jianghan Plain, central China. *Science of The Total Environment* 497–498: 180–187.

Tran N.H., Chen H., Reinhard M., Mao F. & Yew-Hoong Gin K. 2016. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. *Water Research* 104: 461–472.

Tran N.H., Hoang L., Nghiem L.D., Nguyen N.M.H., Ngo H.H., Guo W., Trinh Q.T., Mai N.H., Chen H., Nguyen D.D., Ta T.T. & Gin K.Y.-H. 2019. Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Hanoi, Vietnam. *Science of The Total Environment* 692: 157–174.

Tran N.H., Reinhard M. & Gin K.Y.-H. 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water Research* 133: 182–207.

Tran-Thi N., Lowe R.J., Schurer J.M., Vu-Van T., MacDonald L.E. & Pham-Duc P. 2017. Turning poop into profit: Cost-effectiveness and soil transmitted helminth infection risk associated with human excreta reuse in Vietnam. *PLoS Neglected Tropical Diseases* 11: e0006088.

Udert K., Buckley C., Wächter M., McArdell C., Kohn T., Strande L., Zöllig H., Fumasoli A., Oberson A. & Etter B. 2015. Technologies for the treatment of source-separated urine in the eThekwini Municipality. *Water SA* 41: 212.

UNAIDs. 2023. *Factsheet 2023: Global HIV statistics*. Geneva, Switzerland.

UNEP. 2022. *Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers*. Nairobi, Kenya.

Vergeynst L., Haeck A., De Wispelaere P., Van Langenhove H. & Demeestere K. 2015. Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography–magnetic sector mass spectrometry: Method quality assessment and application in a Belgian case study. *Chemosphere* 119: S2–S8.

Verlicchi P., Al Aukidy M. & Zambello E. 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of The Total Environment* 429: 123–155.

Vieno N.M., Härkki H., Tuhkanen T. & Kronberg L. 2007. Occurrence of Pharmaceuticals in River Water and Their Elimination in a Pilot-Scale Drinking Water Treatment Plant. *Environmental Science & Technology* 41: 5077–5084.

Vieno N.M., Tuhkanen T. & Kronberg L. 2006. Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography-tandem mass spectrometry detection. *Journal of Chromatography A* 1134: 101–111.

Vikesland P.J., Pruden A., Alvarez P.J.J., Aga D., Bürgmann H., Li X., Manaia C.M., Nambi I., Wigginton K., Zhang T. & Zhu Y.-G. 2017. Toward a Comprehensive Strategy to Mitigate Dissemination of Environmental Sources of Antibiotic Resistance. *Environmental Science & Technology* 51: 13061–13069.

Visca A., Caracciolo A.B., Grenni P., Rolando L., Mariani L., Rauseo J., Spataro F., Monostory K., Sperlagh B. & Patrolecco L. 2021. Legacy and emerging pollutants in an urban river stretch and effects on the bacterioplankton community. *Water (Switzerland)* 13: 3402.

Viskari E.-L., Grobler G., Karimäki K., Gorbatova A., Vilpas R. & Lehtoranta S. 2018. Nitrogen Recovery With Source Separation of Human Urine—Preliminary Results of Its Fertiliser Potential and Use in Agriculture. *Frontiers in Sustainable Food Systems* 2: 32.

Wang J. & Wang S. 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. *Journal of Environmental Management* 182: 620–640.

Watkinson A.J., Murby E.J., Kolpin D.W. & Costanzo S.D. 2009. The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. *Science of the Total Environment* 407: 2711–2723.

Werkneh A.A. & Islam M.A. 2023. Post-treatment disinfection technologies for sustainable removal of antibiotic residues and antimicrobial resistance bacteria from hospital wastewater. *Heliyon* 9: e15360.

WHO. 2006. *Guidelines for the safe use of wasterwater excreta and greywater*. Geneva, Switzerland.

WHO. 2022. *Antiretroviral medicines in low- and middle-income countries: forecasts of global and regional demand for 2020-2024.* Geneva, Switzerland.

Wilkinson J.L., Boxall A.B.A., Kolpin D.W., Leung K.M.Y., Lai R.W.S., Galban-Malag C., Adell A.D., Mondon J., Metian M., Marchant R.A., Bouzas-Monroy A., Cuni-Sanchez A., Coors A., Carriquiriborde P., Rojo M., Gordon C., Cara M., Moermond M., Luarte T., Petrosyan V., Perikhanyan Y., Mahon C.S., McGurk C.J., Hofmann T., Kormoker T., Iniguez V., Guzman-Otazo J., Tavares J.L., Figueiredo F.G. de, Razzolini M.T.P., Dougnon V., Gbaguidi G., Traore O., Blais J.M., Kimpe L.E., Wong M., Wong D., Ntchantcho R., Pizarro J., Ying G.G., Chen C.E., Paez M., Martınez-Lara J., Otamonga J.P., Pote J., Ifo S.A., Wilson P., Echeverrıa-Saenz S., Udikovic-Kolic N., Milakovic M., Fatta-Kassinos D., Ioannou-Ttofa L., Belusova V., Vymazal J., Cardenas-Bustamante M., Kassa B.A., Garric J., Chaumot A., Gibba P., Kunchulia I., Seidensticker S., Lyberatos G., Halldorsson H.P., Melling M., Shashidhar T., Lamba M., Nastiti A., Supriatin A., Pourang N., Abedini A., Abdullah O., Gharbia S.S., Pilla F., Chefetz B., Topaz T., Yao K.M., Aubakirova B., Beisenova R., Olaka L., Mulu J.K., Chatanga P., Ntuli V., Blama N.T., Sherif S., Aris A.Z., Looi L.J., Niang M., Traore S.T., Oldenkamp R., Ogunbanwo O., Ashfaq M., Iqbal M., Abdeen Z., O'Dea A., Morales-Saldaña J.M., Custodio M., Cruz H. de la, Navarrete I.,

et al. 2022. Pharmaceutical pollution of the world's rivers. *Proceedings of the National Academy of Sciences of the United States of America* 119: e2113947119.
Wood T.P., Duvenage C.S.J. & Rohwer E. 2015. The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution* 199: 235–243.
Xu W., Pan Z., Wu Y., An X.-L., Wang W., Adamovich B., Zhu Y.-G., Su J.-Q. & Huang Q. 2024. A database on the abundance of environmental antibiotic resistance genes. *Scientific Data* 11: 250.
Yang W., Zhou H. & Cicek N. 2014. Treatment of organic micropollutants in water and wastewater by UV-based processes: A literature review. *Critical Reviews in Environmental Science and Technology* 44: 1443–1476.
Yao L., Wang Y., Tong L., Deng Y., Li Y., Gan Y., Guo W., Dong C., Duan Y. & Zhao K. 2017. Occurrence and risk assessment of antibiotics in surface water and groundwater from different depths of aquifers: A case study at Jianghan Plain, central China. *Ecotoxicology and environmental safety* 135: 236–242.
Zhang S., Abbas M., Rehman M.U., Wang M., Jia R., Chen S., Liu M., Zhu D., Zhao X., Gao Q., Tian B. & Cheng A. 2021. Updates on the global dissemination of colistin-resistant Escherichia coli: An emerging threat to public health. *Science of the Total Environment* 799: 149280.
Zhang H., Du M., Jiang H., Zhang D., Lin L., Ye H. & Zhang X. 2015. Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China. *Environmental Sciences: Processes and Impacts* 17: 225–234.
Zhao H., Zhou J.L. & Zhang J. 2015. Tidal impact on the dynamic behavior of dissolved pharmaceuticals in the Yangtze Estuary, China. *Science of the Total Environment* 536: 946–954.
Zhou X., Cuasquer G.J.P., Li Z., Mang H.P. & Lv Y. 2021a. Occurrence of typical antibiotics, representative antibiotic-resistant bacteria, and genes in fresh and stored source-separated human urine. *Environment International* 146: 106280.
Zhou H., Felipe Beltrán J. & Brito I.L. 2021b. Functions predict horizontal gene transfer and the emergence of antibiotic resistance. *Sci. Adv* 7: 1–12.
Zhuang M., Achmon Y., Cao Y., Liang X., Chen L., Wang H., Siame B.A. & Leung K.Y. 2021. Distribution of antibiotic resistance genes in the environment. *Environmental Pollution* 285: 117402.

ORIGINAL PAPERS

I

OCCURRENCE OF ANTIBIOTICS AND RISK OF ANTIBIOTIC RESISTANCE EVOLUTION IN SELECTED KENYAN WASTEWATERS, SURFACE WATERS AND SEDIMENTS

by

Pius Kairigo, Elijah Ngumba, Lotta-Riina Sundberg,
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Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments

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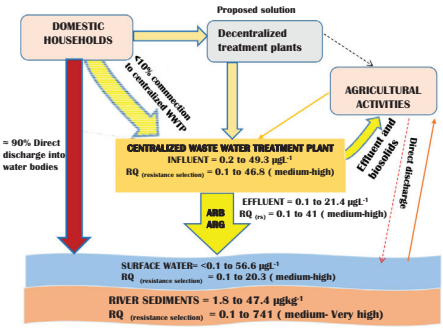
HIGHLIGHTS

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

GRAPHICAL ABSTRACT



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ABSTRACT

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

1. Introduction

Consumption of antibiotics has increased globally (Klein et al., 2018). This is due to increased disease burden, increased availability,

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

The majority of pharmaceuticals do not metabolize completely and therefore are excreted into the environment either in their original form or as pharmacologically active metabolites or transformational products (Carvalho and Santos, 2016). Depending on the category of the compound, 50–90% of ingested APIs are excreted through urine (Kümmerer, 2009; Tran et al., 2016). These APIs and their active metabolites flow into the hydrological cycles by direct discharge into the environment or through wastewater treatment plants (Kümmerer, 2008; Luo et al., 2014; Matongo et al., 2015; Zhang et al., 2015).

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

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

2. Materials and methods

2.1. Study area and sample collection

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

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

2.2. Chemicals and standards

The pharmaceutical standards used were of >99% purity and obtained from Sigma Aldrich (US). The physicochemical properties of the standards, including their structure and CAS registry numbers, are indicated in Table S2 of the supplementary information. All the isotopically labelled internal standards were purchased from Alsachim (France) apart from [$^2H_9$]-TMP which was purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Germany), ammonium hydroxide (25%) solution was purchased from Merck (Belgium), formic acid and formic acid (98%) from Fluka (Germany). Stock solutions were prepared as outlined by Ngumba et al. (2016a, 2016b) and stored at +4 °C in amber vials.

2.3. Sample extraction

200 mL duplicate sub-samples were measured from the pooled sample and 40 μL of 10 mgL^{-1} isotopically labelled mixed standard was added to each before processing. Samples were filtered through a 47 mm GF/F (0.7 μm) glass filter followed by solid-phase extraction using Oasis HLB 6 cc (200 mg) cartridges. The extraction and analytical method developed by Ngumba et al. (2016a, 2016b) was used for the liquid samples. The sample concentration of the target compounds doxycycline (DOX), amoxicillin (AMO), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP) and norfloxacin (NOR) was measured. Target compounds were extracted from the sediment samples using an ultrasonic bath. As outlined by Al-Khazrajy and Alistair (2017), 5 g of the air-dried sediment samples was extracted and the extracts subjected to the solid-phase extraction process. In brief, the HLB cartridges were conditioned with 6 mL of methanol followed by 6 mL of Milli-Q ultrapure water at a flow rate of 5 mL/min^{-1}. Samples spiked with isotopically labelled internal standards were loaded at the same flow rate, after which the target compounds of interest were eluted with 4 mL of 50:50 acetonitrile-methanol solution. The eluting solvent was evaporated under a stream of N_2 gas at 40 °C and the sample reconstituted to 1 mL using 20:80 ACN:H_2O solvent. Filtration was done through a 0.2 μm cellulose acetate membrane filter into HPLC vials ready for analysis.

Table 1
The population, percentage access to improved sources of water, sanitation and sewerage system for the selected sampling areas according to the Kenya National Bureau of 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

source: https://www.knbs.or.ke

2.4. LC-ESI-MS/ MS analysis

A waters alliance 2975 liquid chromatograph (LC, Milford, MA, USA) was used for separation. An Xbridge™ (3.5 μm × 2.1 mm × 100 mm) C_{18} reversed-phase column fitted with a Vanguard® (2.1 mm × 5 mm) pre-column was used. A Quattro micro mass spectrometer (MS) was used for detection. The LC solvent systems and the MS/MS instrument parameters optimized by Ngumba et al. (2016a, 2016b) were used for the targeted multiresidue analysis. The optimized LC-ESI-MS/MS instrument parameters for the analysis of the target compounds are shown in Table S3 of the supplementary information. Figs. S1 and S2 of the supplementary information show the internal standard calibration and the matrix matched calibration graphs, respectively.

2.5. Removal efficiencies

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

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

where C_{Inf} and C_{Eff} refer to the respective measured concentrations ($\mu g L^{-1}$) at the influent and effluent of the WWTP (Sun et al., 2015).

2.6. Risk assessment of antimicrobial resistance selection

The risk quotient (RQ) for antimicrobial resistance selection within the sampled environments was indirectly determined (Tran et al., 2019), according to the measured residual antibiotic concentrations in the representative water samples and the predicted no-effect concentration (PNEC) for resistance selection (RS) as illustrated in Eq. 2. The compound-specific $PNEC_{(RS)}$ values used for risk assessment were proposed by Bengtsson-Palme and Larsson (2016) based on the EUCAST database. The $PNEC_{(RS)}$ values also factored multiple genera of pathogenic microorganisms present in the environment.

$$RQ = \frac{MEC}{PNEC(RS)} \quad (2)$$

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

3. Results

3.1. LC-ESI-MS/MS analysis

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

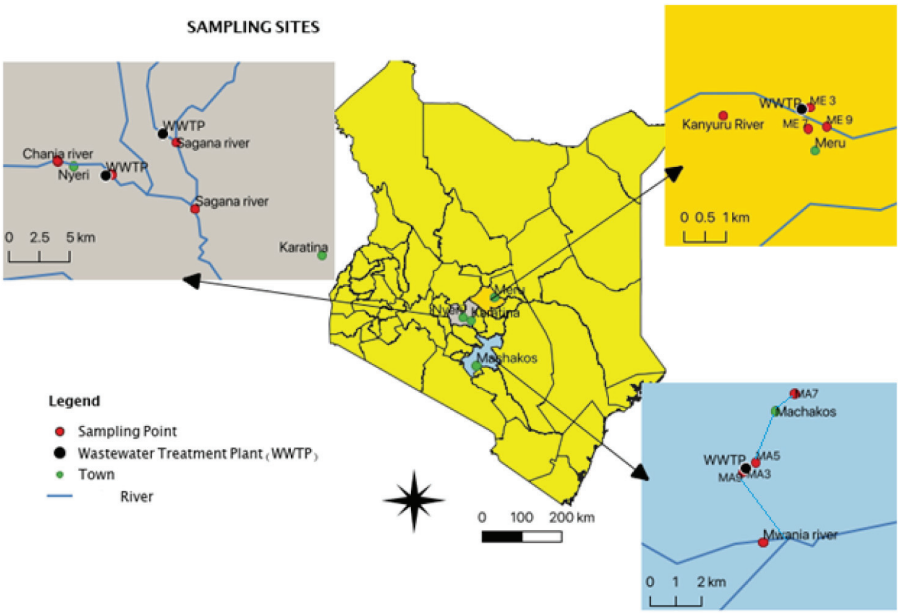

Fig. 1. The map of Kenya and the extrapolated sampling sites in Machakos, Meru and Nyeri.

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

RT = Retention time, SD = Standard deviation, r^2 = linear regression coefficient, LOD = Limit of detection, LOQ = limit of Quantification. (AMO = Amoxicillin, CIP = Ciprofloxacin, TMP = trimethoprim, NOR = Norfloxacin, SMX = Sulfamethoxazole, and DOX = Doxycycline).

3.2. Prevalence of antibiotics and removal efficiency

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

The prevalence of the selected antibiotics was higher in the samples taken from the river sediments than in those from the surface waters. River sediment sample MA9 had SMX, AMO and DOX concentrations of 3.4(0.7), 4.6(0.3) and 8.2(1.3) μgkg^{-1}, respectively, which were considerably 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 at the same location. Similar trend in phase distribution of the antibiotics was recorded in sediment samples NY10, NY12 and NY14 with the following concentration ranges: AMO, 5.9(1.3) to 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}. Sediment sample ME6, which was collected upstream of WWTP4, had higher concentration of AMO, CIP and SMX compared with downstream samples from the same site.

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

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

3.3. Risk assessment of antibiotics for resistance selection

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

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

	Site	Sample type	Code	AMO	CIP	TMP	NOR	SMX	DOX
Machakos	WWTP 1	Influent	MA 1	4.6(0.2)[ab]	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 (200 m upstream)	MA 4	˂LOQ	1.3(0.1)	˂LOQ	0.6(0.01)	49.7(1.5)	0.7(0.1)
		river sediment (200 m upstream)	MA 5	n.d[c]	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 (200 m 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 (1 km 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 warter (2 m downstream)	NY 9	0.3(0.1)	n.d	˂LOQ	0.1(0.03)	0.3(0.05)	n.d
		river sediment(2 m downstream)	NY 10	43.8(3.1)	n.d	˂LOQ	26.0(3.8)	16.3(3.9)	˂LOQ
		Surface water (5 m upstream)	NY 11	˂LOQ	˂LOQ	˂LOQ	˂LOQ	n.d	n.d
		river sediment (5 m upstream)	NY 12	5.9(1.4)	˂LOQ	1.8(0.5)	26.6(3.8)	˂LOQ	32.2(5.7)
		Surface water (200 m)	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 (2 km upstream)	ME 5	˂LOQ	0.24	n.d	n.d	˂LOQ	0.1(0.01)
		river sediment (2 km upstream)	ME 6	11.7(3.2)	47.4(2.8)	˂LOQ	˂LOQ	44.7(3.9)	˂LOQ
		surface grab (500 m downstream)	ME 7	n.d	0.2(0.03)	˂LOQ	˂LOQ	n.d	0.02(0.01)
		river sediment (500 m downstream)	ME 8	˂LOQ	˂LOQ	˂LOQ	˂LOQ	10.4(1.3)	13.9(2.4)
		Surface grab (1 km downstream)	ME 9	n.d	0.2(0.05)	˂LOQ	˂LOQ	n.d	0.1(0.03)
		river sediment(1 km downstream)	ME 10	7.8(1.6)	˂LOQ	˂LOQ	˂LOQ	˂LOQ	11.4(2.1)

[a] Concentration of the analytes reported in μgL^{-1} () standard deviation,$n = 2$.
[b] ˂LOQ - Below Quantification limit.
[c] n.d. - not detected/below limit of detection.

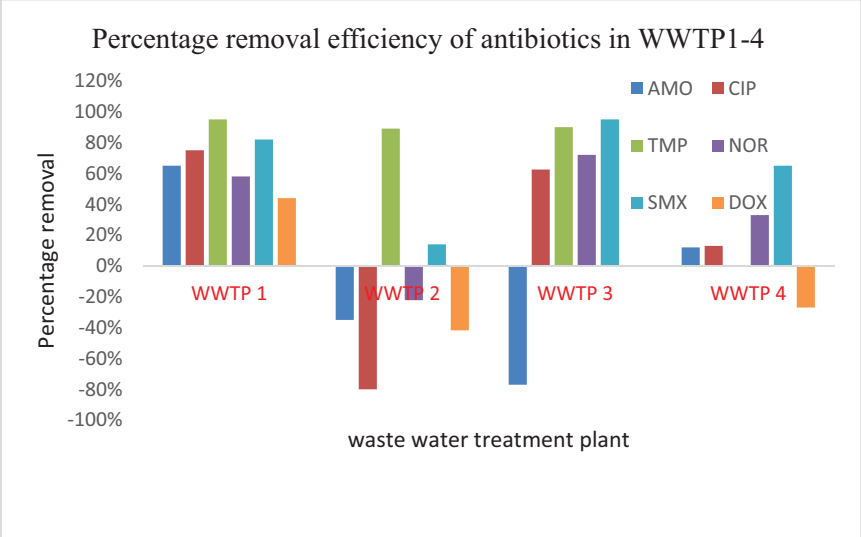

Fig. 2. Percentage removal efficiencies of the antibiotics in the selected treatment plants.

4. Discussion

4.1. Fate of antibiotics in the natural environment

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

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

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

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

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

Negative removal efficiencies for APIs have been reported (K'oreje et al., 2018; Li et al., 2009; Polesel et al., 2016; Thiebault et al., 2017; Udert et al., 2015). Factors causing this may include elimination of antibiotics adsorbed into the particulate matter during sample processing and unaccounted-for hydraulic retention time during sampling. Physicochemical changes during the treatment process influence the adsorption behavior of the antibiotics and hence affect the partition ratio between the aqueous, suspended and sediment phases, and between the influent and effluent concentration (Lindberg et al., 2005). API accumulation, biotic or abiotic dissolution, as well as back transformation and de-conjugation of metabolic products back to parent compounds, can all lead to increased measured concentrations in the effluent relative to the influent (Archer et al., 2017; Haddad et al., 2015; Polesel et al., 2016). SMX transformational products have been shown to back transform to the parent compound under biological and photolytic degradation conditions (Archer et al., 2017; Bagnis et al., 2020). Previous studies in Kenya have reported the presence of 14–112 μgL^{-1} of SMX and 4–20 μgL^{-1} of TMP in wastewater influent, and 10 μgL^{-1} of SMX in the effluent (K'oreje et al., 2018). In addition, two independent studies of the Nairobi river surface water reported SMX concentrations of 13.76μgL^{-1} (Ngumba et al. (2016a, 2016b)) and 23.35 μgL^{-1} (K'oreje et al., 2012) and TMP concentrations 2.65 μgL^{-1} (Ngumba et al. (2016a, 2016b)) and 9.48 μgL^{-1} (K'oreje et al., 2012), respectively. In this article, we report values of the same order of magnitude as other Kenyan studies, but considerably higher than those reported in the global North, as shown in Table 4.

4.2. Risk of evolution of antimicrobial resistance

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

Generally, most of the antibiotics were measured above their compound-specific PNEC values for resistance selection. Increased

Table 4
Previous observations of antibiotic concentrations (μgL^{-1}) in surface waters and urban lakes in different countries and regions. AMO = Amoxicillin, CIP = Ciprofloxacin, TMP = 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. (2016a, 2016b))
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., 2012)
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)
Europe[b]	surface waters	n.r	0.002	0.012	0.004	0.033	(aus der Beek et al., 2016)

n.r = Not reported.
[a] =Concentration range.
[b] =average concentration.

prevalence of antibiotic resistant bacteria could be a result of environmental bacterial communities undergoing resistance selection pressure due to continuous contact with residual antibiotics (Michael et al., 2013; Wu et al., 2018). It has been predicted that resistance resulting from bacterial exposure to subinhibitory concentrations of antibiotics is irreversible, even in the absence of the antibiotic, since the mutants are more stable than the bacteria selected at higher concentrations (Sandegren, 2014). Enrichment and selective advantage of resistant bacteria has also been confirmed at subinhibitory concentrations (Gullberg et al., 2011; Liu et al., 2011).

The use of untreated wastewater for agricultural purposes was observed during sampling. This potentially creates an enormous biosecurity risk by exposing the environment and food chain to residual APIs. Antimicrobial resistance can be transmitted to humans and animals through the food chain, by consumption of untreated water, or indirectly through environmental emissions. Mortality due to drug resistant bacterial infections, like tuberculosis, is on the rise in Kenya, with approximately 169,000 deaths reported in 2017, 30% of which were attributed to multi-drug resistant bacteria (WHO, 2017). Furthermore, 36.7% multidrug resistance among *Klebsiella spp* strains has been reported on the central and western regions of Kenya (Taitt et al., 2017). Further research into AMR at the studied sites is needed.

5. Conclusions

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

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

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

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

CRediT authorship contribution statement

Pius Kairigo: Formal analysis, Methodology, Visualization, Writing - original draft.**Elijah Ngumba:**Data curation, Formal analysis, Methodology, Resources, Software, Validation, Writing - review & editing.**Lotta-Riina Sundberg:**Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.**Anthony Gachanja:**Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.**Tuula Tuhkanen:**Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

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

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

[a] PNEC value corresponds to the size-adjusted lowest MIC divided by an assessment factor of 10 as proposed by Bengston-palme and Larsson (2016).
[b] The number of different bacterial genera and families tested against the specific antibiotic.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137580.

References

Abafe, O.A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., Pietruschka, B., Martincigh, B.S., 2018. LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. Chemosphere 200, 660–670. https://doi.org/10.1016/J.CHEMOSPHERE.2018.02.105.

Al-Khazrajy, O.S.A., Alistair, B.A.B., 2017. Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC-DAD or LC-ESI-MS/MS detection. Anal. Methods 0, 1–11. https://doi.org/10.1039/C7AY00650K.

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12, 465–478. https://doi.org/10.1038/nrmicro3270.

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174, 437–446. https://doi.org/10.1016/J.CHEMOSPHERE.2017.01.101.

aus der Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A., 2016. Pharmaceuticals in the environment-global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823–835. https://doi.org/10.1002/etc.3339.

Bagnis, S., Boxall, A., Gachanja, A., Fitzsimons, M., Murigi, M., Snape, J., Tappin, A., Wilkinson, J., Comber, S., 2020. Characterization of the Nairobi River catchment impact zone and occurrence of pharmaceuticals: implications for an impact zone inclusive environmental risk assessment. Sci. Total Environ. 703. https://doi.org/10.1016/j.scitotenv.2019.134925.

Bengtsson-Palme, J., Larsson, D.G.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ. Int. 86, 140–149. https://doi.org/10.1016/j.envint.2015.10.015.

Carvalho, I.T., Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. Environ. Int. 94, 736–757. https://doi.org/10.1016/j.envint.2016.06.025.

Daughton, C.G., 2016. Pharmaceuticals and the environment (PiE): evolution and impact of the published literature revealed by bibliometric analysis. Sci. Total Environ. 562, 391–426. https://doi.org/10.1016/J.SCITOTENV.2016.03.109.

Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal. Bioanal. Chem. 399, 251–275. https://doi.org/10.1007/s00216-010-4300-9.

Gelband, H., Molly Miller, P., Pant, S., Gandra, S., Levinson, J., Barter, D., White, A., Laxminarayan, R., 2015. Wound healing southern Africa - the state of the world's antibiotics 2015. Wound Heal. South. Africa 8, 30–34.

Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7, e1002158. https://doi.org/10.1371/journal.ppat.1002158.

Guo, J., Selby, K., Boxall, A.B.A., 2016. Assessment of the Risks of Mixtures of Major Use Veterinary Antibiotics in European Surface Waters. https://doi.org/10.1021/acs.est.6b01649.

Haddad, T., Baginska, E., Kümmerer, K., 2015. Transformation products of antibiotic and cytostatic drugs in the aquatic cycle that result from effluent treatment and abiotic/biotic reactions in the environment: an increasing challenge calling for higher emphasis on measures at the beginning of the pipe. Water Res. 72, 75–126. https://doi.org/10.1016/j.watres.2014.12.042.

Hanna, N., Sun, P., Sun, Q., Li, X., Yang, X., Ji, X., Zou, H., Ottoson, J., Nilsson, L.E., Berglund, B., Dyar, O.J., Tamhankar, A.J., Stålsby Lundborg, C., 2018. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: its potential for resistance development and ecological and human risk. Environ. Int. 114, 131–142. https://doi.org/10.1016/J.ENVINT.2018.02.003.

Hirte, K., Seiwert, B., Schüürmann, G., Reemtsma, T., 2016. New Hydrolysis Products of the Beta-Lactam Antibiotic Amoxicillin, their pH-Dependent Formation and Search in Municipal Wastewater. https://doi.org/10.1016/j.watres.2015.11.028.

Ho, L.T., Van Echelpoel, W., Goethals, P.L.M., 2017. Design of waste stabilization pond systems: a review. Water Res. 123, 236–248. https://doi.org/10.1016/J.WATRES.2017.06.071.

Ignatev, A., Tuhkanen, T., 2019. Monitoring WWTP performance using size-exclusion chromatography with simultaneous UV and fluorescence detection to track recalcitrant wastewater fractions. Chemosphere 214, 587–597. https://doi.org/10.1016/j.chemosphere.2018.09.099.

Khan, S., Beattie, T.K., Knapp, C.W., 2017. The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria. Ecotoxicology 26, 283–292. https://doi.org/10.1007/s10646-017-1762-y.

Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc. Natl. Acad. Sci. U. S. A. 115, E3463–E3470. https://doi.org/10.1073/pnas.1717295115.

K'oreje, K.O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J., Van Langenhove, H., 2012. From multi-residue screening to target analysis of pharmaceuticals in water: development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. Sci. Total Environ. 437, 153–164. https://doi.org/10.1016/j.scitotenv.2012.07.052.

K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., Demeestere, K., 2016. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149, 238–244. https://doi.org/10.1016/j.chemosphere.2016.01.095.

K'oreje, K.O., Kandie, F.J., Vergeynst, L., Abira, M.A., Van Langenhove, H., Okoth, M., Demeestere, K., 2018. Occurrence, fate and removal of pharmaceuticals, personal care products and pesticides in wastewater stabilization ponds and receiving rivers in the Nzoia Basin, Kenya. Sci. Total Environ. 637–638, 336–348. https://doi.org/10.1016/j.scitotenv.2018.04.331.

Kronbichler, A., Kerschbaum, J., Gopaluni, S., Tieu, J., Alberici, F., Jones, R.B., Smith, R.M., Jayne, D.R.W., 2018. Trimethoprim–sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis. Ann. Rheum. Dis. 77, 1440–1447. https://doi.org/10.1136/ANNRHEUMDIS-2017-212861.

Kümmerer, K., 2003. Significance of antibiotics in the environment. J. Antimicrob. Chemother. 52, 5–7. https://doi.org/10.1093/jac/dkg293.

Kümmerer, K., 2008. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. https://doi.org/10.1017/CBO9781107415324.004.

Kümmerer, K., 2009. Antibiotics in the aquatic environment – a review – part II. Chemosphere 75, 435–441. https://doi.org/10.1016/J.CHEMOSPHERE.2008.12.006.

Li, B., Zhang, T., Xu, Z., Fang, H.H.P., 2009. Rapid analysis of 21 antibiotics of multiple classes in municipal wastewater using ultra performance liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta 645, 64–72. https://doi.org/10.1016/j.aca.2009.04.042.

Li, D., Zeng, S., He, M., Gu, A.Z., 2016. Water disinfection byproducts induce antibiotic resistance-role of environmental pollutants in resistance phenomena. Environ. Sci. Technol. 50, 3193–3201. https://doi.org/10.1021/acs.est.5b05113.

Lindberg, R.H., Wennberg, P., Johansson, M.I., Tysklind, M., Andersson, B.A.V., 2005. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environ. Sci. Technol. 39, 3421–3429. https://doi.org/10.1021/es048143z.

Liu, A., Fong, A., Becket, E., Yuan, J., Tamae, C., Medrano, L., Maiz, M., Wahba, C., Lee, C., Lee, K., Tran, K.P., Yang, H., Hoffman, R.M., Salih, A., Miller, J.H., 2011. Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. Antimicrob. Agents Chemother. 55, 1204–1210. https://doi.org/10.1128/AAC.01182-10.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

Madikizela, L.M., Tavengwa, N.T., Chimuka, L., 2017. Status of pharmaceuticals in African water bodies: occurrence, removal and analytical methods. J. Environ. Manag. 193, 211–220. https://doi.org/10.1016/j.jenvman.2017.02.022.

Matongo, S., Birungi, G., Moodley, B., Ndungu, P., 2015. Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. Environ. Sci. Pollut. Res. 22, 10298–10308. https://doi.org/10.1007/s11356-015-4217-0.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 47, 957–995. https://doi.org/10.1016/J.WATRES.2012.11.027.

Ngumba, E., Gachanja, A., Tuhkanen, T., 2016a. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River basin, Kenya. Sci. Total Environ. 539, 206–213. https://doi.org/10.1016/J.SCITOTENV.2015.08.139.

Ngumba, E., Kosunen, P., Gachanja, A., Tuhkanen, T., 2016b. A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards. Anal. Methods 8, 6720–6729. https://doi.org/10.1039/c6ay01695b.

Polesel, F., Andersen, H.R., Trapp, S., Plósz, B.G., 2016. Removal of antibiotics in biological wastewater treatment systems - a critical assessment using the activated sludge modeling framework for xenobiotics (ASM-X). Environ. Sci. Technol. 50, 10316–10334. https://doi.org/10.1021/acs.est.6b01899.

Prestinaci, F., Pezzotti, P., Pantosti, A., 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog. Glob. Health 109, 309–318. https://doi.org/10.1179/2047773215Y.0000000030.

Sabri, N.A., Schmitt, H., Van der Zaan, B., Gerritsen, H.W., Zuidema, T., Rijnaarts, H.H.M., Langenhoff, A.A.M., 2018. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. J. Environ. Chem. Eng., 102245 https://doi.org/10.1016/j.jece.2018.03.004.

Sandegren, L., 2014. Selection of antibiotic resistance at very low antibiotic concentrations. Ups. J. Med. Sci. 119, 103–107. https://doi.org/10.3109/03009734.2014.904457.

Sobsey, M.D., Abebe, L., Andremont, A., Ashbolt, N.J., Husman, A.M. de R., Gin, K.Y.-H., Hunter, P.R., Meschke, J.S., Vilchez, S., 2014. Briefing note antimicrobial resistance : an emerging water. Sanitation and Hygiene (16) https://doi.org/10.13140/RG.2.2.24776.32005.

Sun, Q., Li, M., Ma, C., Chen, X., Xie, X., Yu, C.-P., 2015. Seasonal and Spatial Variations of PPCP Occurrence, Removal and Mass Loading in Three Wastewater Treatment Plants Located in Different Urbanization Areas in Xiamen, China. https://doi.org/10.1016/j.envpol.2015.10.003.

Taitt, C.R., Leski, T.A., Erwin, D.P., Odundo, E.A., Kipkemoi, N.C., Ndonye, J.N., Kirera, R.K., Ombogo, A.N., Walson, J.L., Pavlinac, P.B., Hulseberg, C., Vora, G.J., 2017. Antimicrobial resistance of Klebsiella pneumoniae stool isolates circulating in Kenya. PLoS One 12, 1–19. https://doi.org/10.1371/journal.pone.0178880.

Thiebault, T., Boussafir, M., Le Milbeau, C., 2017. Occurrence and removal efficiency of pharmaceuticals in an urban wastewater treatment plant: mass balance, fate and consumption assessment. J. Environ. Chem. Eng. 5, 2894–2902. https://doi.org/10.1016/j.jece.2017.05.039.

Tran, N.H., Chen, H., Reinhard, M., Mao, F., Yew-Hoong Gin, K., 2016. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. Water Res. 104, 461–472. https://doi.org/10.1016/j.watres.2016.08.040.

Tran, N.H., Hoang, L., Nghiem, L.D., Nguyen, N.M.H., Ngo, H.H., Guo, W., Trinh, Q.T., Mai, N.H., Chen, H., Nguyen, D.D., Ta, T.T., Gin, K.Y.-H., 2019. Occurrence and risk assessment of multiple classes of antibiotics in urban canals and lakes in Hanoi, Vietnam. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.07.092.

Udert, K., Buckley, C., Wächter, M., McArdell, C., Kohn, T., Strande, L., Zöllig, H., Fumasoli, A., Oberson, A., Etter, B., 2015. Technologies for the treatment of source-separated urine in the eThekwini municipality. Water SA 41, 212. https://doi.org/10.4314/wsa.v41i2.6.

Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., Laxminarayan, R., 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect. Dis. 14, 742–750. https://doi.org/10.1016/S1473-3099(14)70780-7.

Walker, A., Ford, D., Gilks, C., Munderi, P., Ssali, F., Reid, A., Katabira, E., Grosskurth, H., Mugyenyi, P., Hakim, J., Darbyshire, J., Gibb, D., Babiker, A., 2010. Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort. Lancet 375, 1278–1286. https://doi.org/10.1016/S0140-6736(10)60057-8.

WHO, 2016. WHO | Antimicrobial Resistance: Global Report on Surveillance 2014 (WHO).

WHO, 2017. Global Tuberculosis Report 2017.

Wu, J., Huang, Y., Rao, D., Zhang, Y., Yang, K., 2018. Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. Front. Microbiol. 9, 745. https://doi.org/10.3389/fmicb.2018.00745.

Zhang, H., Du, M., Jiang, H., Zhang, D., Lin, L., Ye, H., Zhang, X., 2015. Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River basin, South China. Environ. Sci. Process. Impacts 17, 225–234. https://doi.org/10.1039/c4em00457d.

SUPPLEMENTARY DATA

I

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

by

Pius Kairigo, Elijah Ngumba, Lotta-Riina Sundberg, Anthony Gachanja & Tuula Tuhkanen 2020

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SUPPLEMENTARY MATERIAL

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

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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

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)	$C_{22}H_{24}N_2O_8$	564-25-0	630	70	
Amoxicillin (AMO)	$C_{16}H_{19}N_3O_5S$	26787-78-0	958	60-80	
Sulfamethoxazole(SMX)	$C_{10}H_{11}N_3O_3S$	723-46-6	610	15-25	0.89
			400	80-90	0.91
Trimethoprim (TMP)	$C_{14}H_{18}N_4O_3$	738-70-5			
	$C_{17}H_{18}FN_3O_3$		80	80	0.28
Ciprofloxacin (CIP)		85721-33-1			
Norfloxacin (NOR)	$C_{16}H_{18}N_3O_3F$	70458-96-7	13500	60	

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

Table S3: Optimized LC-ESI-MS/MS instrument parameters for the analysis of the target 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	$[^2H_8]$-CIP	2.24 (0.08)	332.1 (34)	288.0 (19)	314.1 (19)
TMP	$[^2H_9]$TMP	2.25 (0.05)	291.1 (34)	123.0 (19)	230.0 (19)
NOR	$[^2H_8]$-NOR	2.15 (0.06)	320.3 (30)	276.0 (18)	302.0 (25)
SMX	$[^2H_4]$-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)

[a]ILIS isotopically labelled internal standard. [b]RT retention time. [c]CV collision voltage [d]CE collision energy. n.a not available[1]

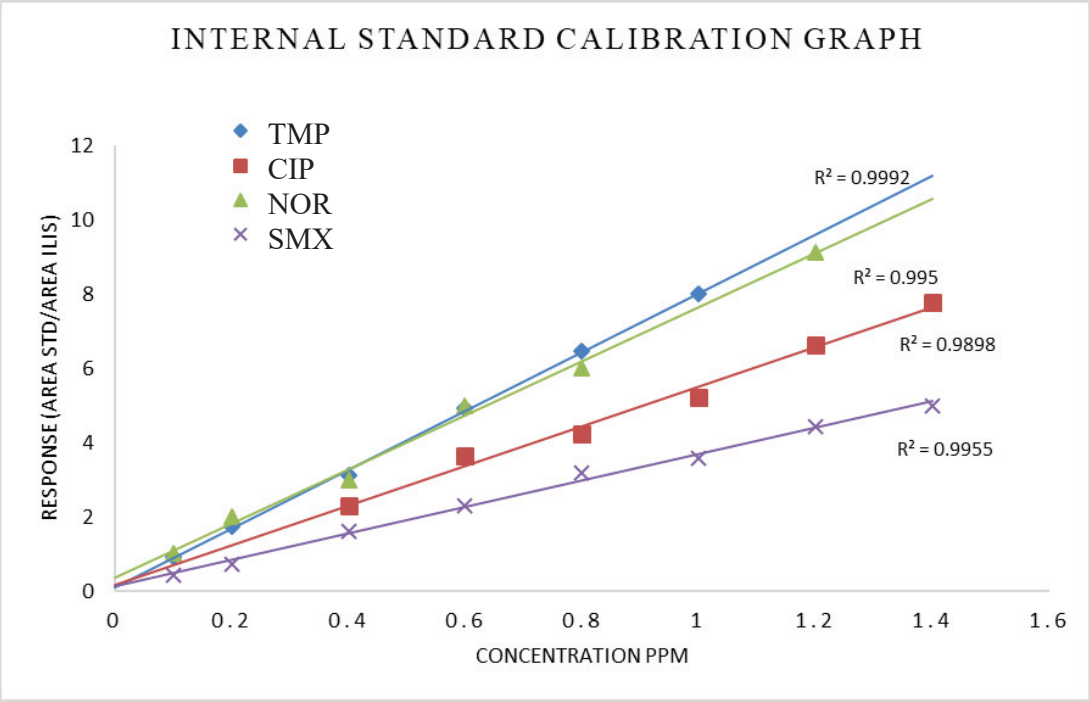

Figure S1: Calibration graph for TMP, CIP, NOR and SMX constructed by plotting the ratio of the Area of the standard divided by the area of the isotopically labelled internal standard against the concentration.

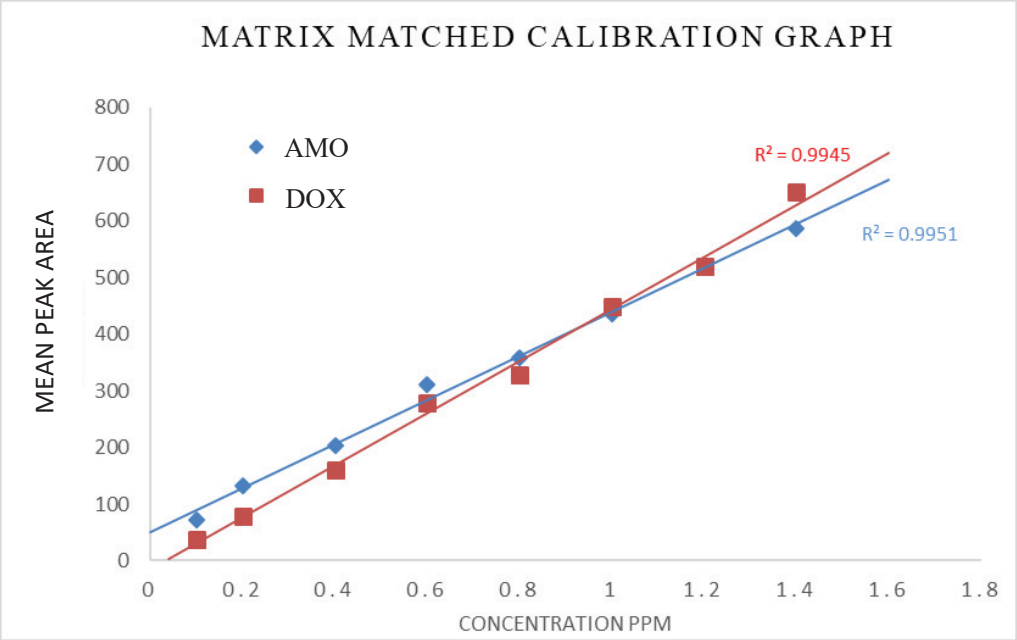

Figure S2: Matrix matched calibration graph for AMO and DOX constructed by spiking surface water at concentration levels between 0ppm (blank) and 1.4ppm

References

1 L. M. Madikizela, N. T. Tavengwa and L. Chimuka, *J. Environ. Manage.*, 2017, **193**, 211–220.

2 E. Ngumba and T. Anthony, Gachanja; Tuhkanen, *Sci. Total Environ.*, 2016, **539**, 206–213.

3 K. O. K'oreje, L. Vergeynst, D. Ombaka, P. De Wispelaere, M. Okoth, H. Van Langenhove and K. Demeestere, *Chemosphere*, 2016, **149**, 238–244.

4 Drugbank www.drugbank.ca

II

CONTAMINATION OF SURFACE WATER AND RIVER SEDIMENTS BY ANTIBIOTIC AND ANTIRETROVIRAL DRUG COCKTAILS IN LOW AND MIDDLE-INCOME COUNTRIES: OCCURRENCE, RISK AND MITIGATION STRATEGIES

by

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Article

Contamination of Surface Water and River Sediments by Antibiotic and Antiretroviral Drug Cocktails in Low and Middle-Income Countries: Occurrence, Risk and Mitigation Strategies

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Abstract: Presence of antimicrobial cocktails in the hydrological cycles is of interest because of their potential to mediate antimicrobial resistance within the natural environment. In this study, we determined the concentrations of selected antibiotics and antiretroviral drugs (ARVDs) in wastewater treatment plant (WWTP) effluent, effluent suspended particulate matter (SPM), surface waters and river sediments in Kenya in order to determine the extent of pollution within the sampled environment. Target analysis for the most common antibiotics and ARVDs was done. Sulfamethoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR), zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) were analyzed using LC-ESI-MS/MS. Effluent aqueous phase had concentrations ranging between 1.2 μg L^{-1} to 956.4 μg L^{-1} while the effluent SPM showed higher concentrations, ranging between 2.19 mg Kg^{-1} and 82.26 mg Kg^{-1}. This study shows emission of active pharmaceutical ingredients (APIs) from WWTP to the environment mainly occurs via the SPM phase, which is usually overlooked in environmental analyses. Concentrations in surface waters and river sediments ranged between 1.1 μg L^{-1} to 228 μg L^{-1} and 11 μg Kg^{-1} to 4125 μg Kg^{-1} respectively. ARVDs occurred at consistently higher concentrations than antibiotics in both the aqueous and solid samples. The wastewater treatment plants and lagoons where sludge degradation should occur, are sources of active pharmaceutical ingredients (APIs) including transformational products, nutrients and organic matter that are released back to the aqueous phase.

Keywords: wastewater; antibiotics; antiretroviral drugs; antimicrobial resistance; suspended particulate matter; sediments

1. Introduction

Pollution by pharmaceutical micropollutants is an emerging area of concern. The effect of cocktails of active pharmaceutical ingredients (APIs) to non-target organisms is largely unknown [1]. Cocktails of APIs and their active metabolites enter the environment due to incomplete removal by wastewater treatment plants (WWTPs) after human and veterinary use. Indeed, centralized wastewater treatment plants are point sources of emerging micropollutants, especially active pharmaceutical ingredients (APIs) into the environment [2]. This happens because pharmaceuticals are not completely metabolized in the body and are excreted in urine and fecal matter, as either parent compounds

or as pharmacologically active metabolites [3–6]. In water-based sanitation, the active ingredients and their metabolites undergo dilution with large volumes of water as they are flushed down the drain, where they mix with other household chemicals and personal care products. Household, hospital and industrial wastewater as well as a runoff water mix and are channeled into the centralized WWTPs. Dilution of the organic micropollutants to very low concentrations (ng L^{-1} or below) occurs, which cannot be effectively removed from the WWTP, making them ubiquitously present in the water bodies [7]. Pseudo-persistent APIs in the environment have the potential to mediate antimicrobial resistance among the environmental pathogenic microorganism [8]. Effluent from healthcare facilities, WWTPs, pharmaceutical and other industries—especially in low- and middle-income countries—is insufficiently regulated [9]. WWTPs are beneficial for public health; however, they act as sinks to important nutrients such as phosphorus and nitrogen as well as minerals. In wastewater, plants where the activated sludge removal, treatment and discharge into landmines or fields occurs, the flow of adsorbed recalcitrant micropollutants happens between aqueous phase to terrestrial systems. Wastewater treatment plants such as lagoons, anaerobic and aerobic ponds and trickling filters in which the excess sludge is meant to decompose, are where the nutrients and recalcitrant matter including micropollutants are desorbed and released from the sludge into the effluent and eventually into the receiving water bodies, where they potentially cause eutrophication and stress to aquatic organisms [10,11].

Previous research work on this topic has focused on the aqueous phase, but here we also report data on occurrence of active pharmaceutical ingredient (API) cocktails in suspended particulate matter (SPM) and river sediments. This study was aimed at assessing the levels of selected common antibiotics; sulfametoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR) and antiretroviral drugs; zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) in the effluent, SPM surface water and river sediments of selected sampling sites in Kenya.

2. Materials and Methods

2.1. Study Area and Sample Collection

Effluent surface water grab and river sediment upstream and downstream of the effluent discharge point from the wastewater treatment plant (WWTP) was collected in Machakos town, Kenya. The WWTP Machakos in Machakos employs waste stabilization ponds for wastewater treatment. Machakos town is the administrative town of the larger Machakos County. The Machakos town constituency has a population of 50,753, with a WWTP serving 7.6% of the population while 13.1% and 55.2% of the population use septic tanks and pit latrines, respectively [12].

Two sampling campaigns in January and September 2019 were carried out. September is usually a very dry month and most arid and semi-arid areas suffer drought, affecting the flow rates into the treatment plant as well as in the rivers. The river Mitheu, which receives the effluent from the WWTP, was almost drying up and the flowing waters were contaminated with raw sewage judging by the odor and appearance. Generally, the water volumes in the rivers were significantly decreased during the September sampling as compared to the January sampling. The physicochemical characteristics of the samples are shown in Table 1. The effluent composite sample was constituted by combining the eight hourly grab samples into a large container from which duplicate 1-L representative samples were drawn and taken to the laboratory for further processing. Duplicate grab 1-L river water samples were collected at approximately 500 m upstream and downstream of the effluent discharge point. River sediment samples were collected at similar points corresponding to the aqueous samples and dried indoors at room temperature (25 °C). The sample collection protocols are described in detail in our previous publication [13]. The suspended particulate matter was obtained by successive filtration of 100 mL of the aqueous sample through a Whatman GF/D (2.7 μm) and GF/F (0.7 μm) filter papers. The filter papers were dried at room temperature (25 °C) and processed similarly to the sediment samples.

Table 1. Psychochemical characteristics of the effluent and surface water samples showing the pH, temperature, electrical conductivity, total dissolved solids and total suspended solids. ES = electrical conductivity, TDS = total dissolved solids and TSS = total suspended solids.

Sample	pH	Temp (°C)	EC (dS/m)	TDS (ppm)	TSS (mg L^{-1})
Effluent	7.88	30.2	5610	3.73	72.8
Surface water	6.36	27.6	1140	2.86	66.4

2.2. Chemicals and Standards

All pharmaceutical standards and corresponding isotope-labelled internal standards were of >99% purity. All the isotopically labeled internal standards were purchased from Alsachim (Illkirch Graffenstaden, France) apart from ($^2H_9$)-TMP which was purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), ammonium hydroxide (25%) solution was purchased from Merck (Overijse, Belgium), formic acid and formic acid (98%) from Fluka (Munich, Germany). Stock solutions and the working standards were prepared and stored at +4 °C in amber vials.

2.3. Sample Cleanup and Pre-Concentration

Environmental sample cleanup and pre-concentration for aqueous samples was carried out following the protocol described by Ngumba et al., (2016) [14] without modifications. The river sediment samples were analyzed by the method described elsewhere with some modifications [15].

Briefly, 1 g of dried sediment was weighed into a 50 mL centrifuge tube (VWR), spiked with 40 µL of 10 mg L^{-1} mixture of isotopically labeled internal standards, and allowed to equilibrate for ~30 min at room temperature. Extracting solvent (methanol:water, 80:20) was added (6 mL) to the mixture and vortexed for one minute. The mixture was sonicated for 20 min using a ultrasonic bath sonicator, VWR USC 1200TH, Leicestershire, UK. Extracts were centrifuged at 4500 rpm with SANYO HARRIER18/80, London, UK for 10 min and the supernatant collected in a 15 mL glass Kimax® test tubes. A repeat extraction using 6 mL of 100% methanol was done and extracts were combined into the 15 mL tube. Evaporation under a stream of nitrogen to approximately 1 mL followed and reconstituted to 10 mL using milli-Q water. The reconstituted sample cleanup followed the protocol described by Ngumba et al., 2016 [16] for surface and wastewater samples.

2.4. Instrumental Analysis

An isotope dilution method was employed in the analysis of all the target compounds. Eight-point calibration curves were prepared for each analyte by plotting response ratio of the peak area of analyte divided by peak area of internal standard (*y*-axis) against concentration ratio of the analyte divided by concentration of internal standard (*x*-axis). The multiple reaction monitoring parameters are shown in Table 2.

APIs were analyzed using a Quattro micro tandem mass spectrometer interfaced with a waters alliance 2975 liquid chromatograph (LC, Milford, MA, USA). The C_{18} reversed-phase column used was (3.5 µm × 2.1 mm × 100 mm Xbridge™) fitted with a 2.1 mm × 5 mm Vanguard® and pre-column was used for separation. Gradient elution method with Formic acid (0.1%) in water and acetonitrile (100%) was used as the mobile phase. Multiple reaction monitoring (MRM) in positive ion mode was used for the determination of the analytes. The multiresidue method for trace level analysis of antibiotics and antiretroviral drugs previously published in our research group by Ngumba, Kosunen et al. (2016) [16] was used without modification.

Table 2. The multiple reaction monitoring parameters. SMX = sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine.

Target Compound	RT [a]	Precursor Ion $[M + H]^+$ (m/z) (CV) [b]	Quantifier Ion (m/z) (CE) [c]	Qualifier Ion (CE)
3TC	1.5	229.9 (17)	112.0 (18)	95.0 (29)
ZDV	2.3	268.2 (16)	127.0 (17)	110.1 (25)
NVP	4.1	267.2 (40)	226.2 (29)	198 (29)
CIP	2.2	332.1 (34)	288.0 (19)	314.1 (19)
TMP	2.2	291.1 (34)	123.0 (19)	230.0 (19)
NOR	2.1	320.3 (30)	276.0 (18)	302.0 (25)
SMX	5.1	254.0 (28)	156.0 (18)	108.0 (17)

[a] RT retention time. [b] CV collision voltage [c] CE collision energy.

3. Results

3.1. Instrumental Analysis Results

Table 3 shows the LC-MS/MS-ESI method qualification results. All the target compounds were detected in all the samples with the limit of detection ranging between 3 ng L^{-1} and 18 ng L^{-1}.

Table 3. LC-MS/MS-ESI method qualification results. API = active pharmaceutical ingredient, ILIS = isotopically labelled internal standard, DF = detection frequency, LOQ = limit of quantification, SMX = sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine.

API	ILIS	r^2	% Recovery (RSD)	DF (%)	LOQ ng L^{-1}
NOR	($^2H_8$)-CIP	0.996	92.6 (3.2)	100	12
TMP	($^2H_9$)-TMP	0.999	111.3 (4.1)	100	9
CIP	($^2H_8$)-CIP	0.993	84.3 (8.3)	100	10
SMX	($^2H_4$)-SMX	0.997	101 (7.2)	100	17
3TC	($^{13}C^2H_2$ $^{15}N_2$)-3TC	0.993	98.8 (3.7)	100	15
ZDV	($^{13}C^2H_3$)-ZDV	0.988	98.7 (19.4)	100	53
NVP	($^2H_4$)-NVP	0.989	87.7 (9.3)	100	19

3.2. Occurrence of API Cocktails in the Effluent, SPM, Surface Water and River Sediments

Prevalence of antibiotics and antiretroviral drug cocktails in the effluent, effluent SPM, surface waters and sediments are shown Table 4, respectively. In the antibiotic category, SMX was predominant in the aqueous phase with a concentration range of 96 µg L^{-1} and 142 µg L^{-1} measured approximately 500 m upstream and downstream to the effluent discharge point. ARVDs were also ubiquitously present in the aqueous samples with 3TC occurring twice as much as SMX in the surface waters. The concentration of APIs in the effluent discharged into the river ranged between 1.4 µg L^{-1} and 956.4 µg L^{-1} with 3TC and SMX having the highest concentration. APIs in the effluent SPM and the river sediments occurred in µg kg^{-1} to mg kg^{-1} levels as shown in Table 4. These results indicate the effluent SPM is the major pathway for emission of APIs from the WWTP into the receiving water.

Data from the two sampling campaigns showed significant variability, with SMX and 3TC dominating. This could mainly be attributed to the drought situation during the September sampling, whereby the receiving river was almost drying up. The seasonal variation of the January and September sampling campaign results are illustrated in Figure 1.

Table 4. Prevalence of antibiotics and antiretroviral cocktails in effluent, SPM, surface water and river sediments in the September sampling. SPM = suspended particulate matter, SMX = Sulfamethoxazole, CIP = ciprofloxacin, TMP = trimethoprim, NOR = norfloxacin, ZDV = zidovidine, 3TC = lamivudine, NVP = nevirapine. (sd, n = 3) PNEC = compound specific predicted no effect concentration for antimicrobial resistance selection, n.a = Not available.

Compound	Effluent Aqueous Phase	Effluent SPM Phase	Water μg L^{-1}		Sediments μg kg^{-1}		PNEC [15]
	μg L^{-1}	μg kg^{-1}	Upstream 500 M	Downstream 500 M	Upstream 500 M	Downstream 500 M	μg L^{-1}
NOR	4.2 (0.8)	82,267 (559)	1.6 (0.4)	4.9 (1.2)	776 (22)	248 (35)	0.5
TMP	15.8 (1.1)	3080 (845)	3.8 (1.2)	4.4 (1.5)	11 (3.2)	90 (19)	0.5
CIP	5.3 (0.6)	5017 (344)	2.5 (0.9)	2.8 (1.1)	4125 (236)	1275 (67)	0.064
SMX	956.4 (9.4)	23,448 (1959)	96.9 (4.6)	142.6 (8.3)	542 (13)	896 (25)	16
3TC	847.1 (25.3)	69,681 (5824)	219.6 (16.9)	228.3 (11)	491 (18.2)	107 (12)	n.a
ZDV	1.4 (1)	3336 (119)	2.1 (1.3)	1.1 (0.9)	510 (40)	118 (18)	n.a
NVP	9.5 (2.2)	3214 (146)	0.9 (0.4)	2.3 (1)	95 (14)	101 (11)	n.a

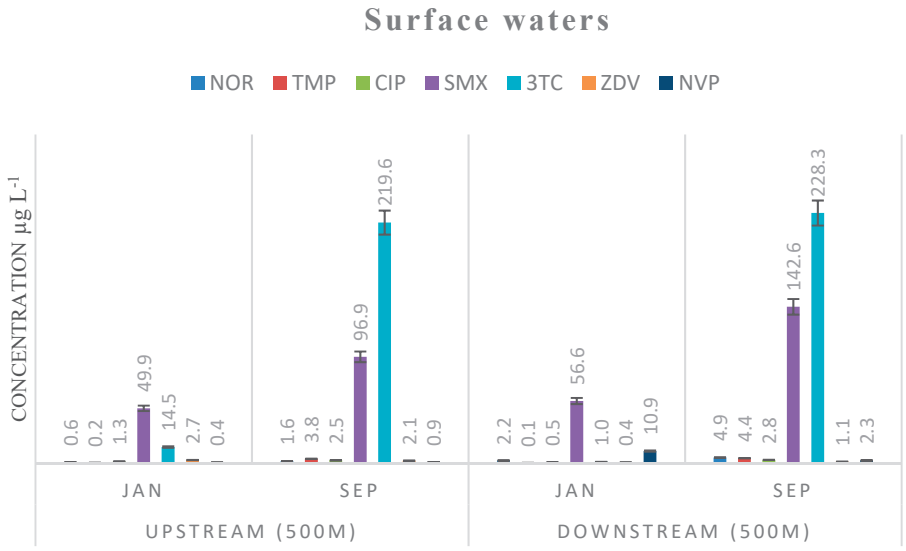

Figure 1. Seasonal variation of APIs in surface waters.

4. Discussion

4.1. Cocktails of APIs in the Natural Environment within Low- and Medium-Income Countries

This study confirms the presence of antibiotic and ARVD cocktails in the environmental samples. Concentrations of sulfamethoxazole (SMX), ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR), zidovidine (ZDV), lamivudine (3TC) and nevirapine (NVP) in surface water and sediments were analyzed using a robust LC-ESI-MS/MS method. Ubiquitous detection of the all target APIs in all the collected representative samples was reported. This indicates the emission of substantial amounts of the residual antibiotics and ARVDs into the environment within the sampling areas and especially through the SPM. The measured concentrations of APIs upstream of the WWTP discharge point signifies non-point loading. Furthermore, effluent from WWTPs are considered point sources of APIs because they cannot completely remove pharmaceuticals and other personal care products within the treatment process [17,18]. Sorption of API to the SPM within the WWTP effluent is a pathway of emission of hydrophobic micropollutants to surface waters and river sediments. Active ingredients adsorbed into the SPM enriches the sediments. Resuspension of adsorbed compounds into the aqueous phase due to biotic and abiotic activity can maintain pseudo persistency of organic micropollutants [19,20].

This can particularly occur in WWTPs where removal of sludge does not exist. The accumulated and formed sludge decomposes and the nutrients and recalcitrant matter ends up as effluent and thus

spills into the receiving water bodies [21]. Other point sources include directly discharged human waste into the water bodies, because ingested drugs do not metabolize fully in the body and excretion occurs in urine and fecal matter as a parent compound or active metabolites [22,23].

The relatively higher concentration levels of APIs measured upstream of the effluent discharge point compared to the downstream samples could be attributed to the direct discharge of untreated wastewater into water bodies from informal settlements, illegal health clinics or from veterinary use. Furthermore, effluent from the WWTP is a major emission source of API into the receiving waters, with the SPM phase accounting for the bulk of the APIs emitted into the receiving waters as compared to the aqueous phase. Reduced flow rates of the surface waters due to drought in the sampling area in September indicates a lack of sufficient dilution of the WWTP effluent, thereby recording relatively high concentrations downstream of the discharge point. In most of the Kenyan towns, the centralized sewerage system covers 7.6% of the population, with the rest of the population using other sanitation solutions such as pit latrines [12]. Rapidly developing informal settlements within urban towns without a proper sanitation system increases the probability of discharge of raw sewage to surface waters.

HIV and AIDS remain a major public health issue of concern with an estimated 770,000 global fatalities in 2018. Out of the approximate 37.9 million people living with HIV/AIDS worldwide, 25.7 million are in the African region, out of which 16.3 are on lifelong antiretroviral therapy (ART). As of 2018, Kenya had approximately 1.49 million patients of which 75% were on ART, while in the same period South Africa had 7.7 million patients with approximately 62% of adults on ART. [24,25]. In 2015, it was estimated that 159,000 Kg of ARVDs reach water bodies annually in South Africa [26]. Prevalence of ARVDs in South African surface waters, ranging between 0.407 μg L^{-1} to 0.973 μg L^{-1} [27], 0.003 μg L^{-1} to 0.0067 μg L^{-1} [26] and 0.0046 μg L^{-1} to 34 μg L^{-1} [28] was reported.

Recent studies done in Kenyan surface waters have reported concentration values ranging from 6 μg L^{-1} to 167 μg L^{-1} [29], 0.5 to 1 μg L^{-1} [30] and 0.5 μg L^{-1} to 7.6 μg L^{-1} [14] for ZDV, NVP and 3TC. These results were in the same order of magnitude as the results reported in this study. Although environmental data on residual API is still scanty in developing countries, results reported by other recent studies done elsewhere on the African continent are shown in Table 5.

Table 5. Occurrence of antibiotic and antiretroviral drug residues in selected African surface waters and WWTP effluents. <LOQ = below limit of quantification, <MQL = below method quantification limit, n.d. = not detected.

Category	Compound	Sample	Concentration Range μg L^{-1}	Country	Ref.
Antibiotics	Sulfamethoxazole	surface waters	<LOQ to 9.64	Ghana	[31]
		surface waters	<LOQ to 49.56	Kenya	
		surface waters	0.511 to 53.83	Mozambique	
		surface waters	0.0033 to 10.57	South Africa	
		surface waters	11.25	Kenya	[32]
		effluent/surface water	<MQL to 0.019	Egypt	[33]
		surface water	<0.01 to 1.5	Nigeria	[34]
	Trimethoprim	surface waters	0.014 to 1.37	Ghana	[31]
		surface waters	<LOQ to 11.38	Kenya	
		surface waters	0.31 to 6.22	Mozambique	
		surface waters	0.004 to 5.88	South Africa	
		surface water	3.35	Kenya	[32]
		surface water	<0.01 to 0.4	Nigeria	[34]
		effluent/surface water	0.21 to 1.06	Egypt	[33]
	Ciprofloxacin	surface water	0.51 to 14.33	South Africa, Ghana, Kenya	[17]

Table 5. *Cont.*

Category	Compound	Sample	Concentration Range μg L^{-1}	Country	Ref.
ARVDs	Zidovudine	effluent/surface water	n.d. to 5.3	South Africa	[35]
		effluent	12.1 to 20.13	Kenya	[36]
	Nevirapine	effluent/surface water	<LOQ to 0.28	South Africa	[35]
		effluent	0.0053 to 3.3	Kenya	[36]
	Lamivudine	effluent/surface water	0.13 to 20.93	South Africa	[35]
		effluent	0.0325 to 60.68	Kenya	[36]

4.2. Risk of APIs in the Environment

Measured environmental concentrations of APIs shown are above the compound-specific no-effect concentrations and thus can affect non-target environmental microorganisms and aquatic life [13]. This could result in mediation of resistance selection in pathogenic microorganisms within the natural environment, resulting in antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG). WWTPs were identified as point sources of ARBs and ARGs [37]. Antimicrobial resistance is a threat to global public health and can affect anybody in any part of the world. Resistant pathogens developed in the natural environment are harder to treat using available antimicrobials, and hence their infections can lead to an increased cost of treatment, lengthy hospitalization periods and eventually death. Pharmaceutical mixtures within the environment can have additive effects even though the risk of individual compounds could be negligible. For instance, antibiotic drug combinations designed to work synergistically, such as TMP-SMX (co-trimoxazole) with a combination ratio of 1:5 [38]. These combination ratios can also exist within natural environments, where their synergistic activity continues to act in the environmental microorganism, a precursor for antimicrobial resistance selection. Measured environmental concentrations in this study were consistently higher in the river sediment phase as compared to the surface water. This could mean that the risk of resistance selection could be greatest in the sediment phase [14]. These phases were commonly overlooked in previous studies.

Similarly to bacteria, viruses can evolve resistance against antiviral drugs, especially in instances where there is a co-existence of the virus to be treated with the antiviral drug [36]. More studies on the development of antiviral resistance is needed. Resistant infections kill approximately 58,000 newborn children in India every year [39]. Over 2.8 million resistant infections occur yearly in the United States of America, resulting in over 35,000 deaths each year [40]. In Kenya, approximately 50,000 people die each year due to multidrug-resistant tuberculosis [41]. At a time when antimicrobial resistance causes major problems in healthcare and new viral diseases emerge, it is central to understand antimicrobial contamination in the environment.

5. Conclusions

This study determined the prevalence and concentration of antibiotic and antiretroviral drug cocktails in the effluent, SPM, surface waters and river sediments of selected sampling areas in Kenya. To the best of our knowledge, this is the first study to report the occurrence of APIs in the SPM phase within Kenyan WWTP effluents. The results indicate that SPM is an important phase for consideration in the determination of emission of micropollutants from WWTPs. Surface waters and sediments were found to be contaminated with elevated levels of the target compounds. APIs in the environment can have effects on public health on a global scale. Decentralized sanitation solutions, especially in informal settlements in the peri-urban areas, can help mitigate the direct discharge of raw sewage into surface waters. Sustainable sanitation solutions aimed at separating the urine at source are recommended, since urine is a point source of human pharmaceuticals.

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References

1. Vasquez, M.I.; Lambrianides, A.; Schneider, M.; Kümmerer, K.; Fatta-Kassinos, D. Environmental side effects of pharmaceutical cocktails: What we know and what we should know. *J. Hazard. Mater.* **2014**, 169–189. [CrossRef] [PubMed]
2. Kümmerer, K. *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*; Springer: Berlin, Germany, 2008; Volume 1. [CrossRef]
3. Kümmerer, K. Antibiotics in the aquatic environment—A review—Part I. *Chemosphere* **2009**, *75*, 417–434. [CrossRef] [PubMed]
4. Tambosi, J.L.; Yamanaka, L.Y.; José, H.J.; De Fátima Peralta Muniz Moreira, R.; Schröder, H.F. Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP). *Quim. Nova* **2010**, *33*, 411–420. [CrossRef]
5. Tran, N.H.; Chen, H.; Reinhard, M.; Mao, F.; Yew-Hoong Gin, K. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. *Water Res.* **2016**, *104*, 461–472. [CrossRef] [PubMed]
6. Carvalho, I.T.; Santos, L. Antibiotics in the aquatic environments: A review of the european scenario. *Environ. Int.* **2016**, *94*, 736–757. [CrossRef] [PubMed]
7. Pereira, A.; Silva, L.; Laranjeiro, C.; Lino, C.; Pena, A. Selected pharmaceuticals in different aquatic compartments: Part I—Source, fate and occurrence. *Molecules* **2020**, *25*, 1026. [CrossRef]
8. Gullberg, E.; Cao, S.; Berg, O.G.; Ilbäck, C.; Sandegren, L.; Hughes, D.; Andersson, D.I. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* **2011**, *7*, e1002158. [CrossRef]
9. Yakubu, O.H. Pharmaceutical wastewater effluent-source of contaminants of emerging concern: Phytotoxicity of metronidazole to soybean (Glycine Max). *Toxics* **2017**, *5*, 10. [CrossRef]
10. Sharma, V.K.; Johnson, N.; Cizmas, L.; McDonald, T.J.; Kim, H. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere* **2016**, *150*, 702–714. [CrossRef]
11. Larsen, T.A.; Maurer, M.; Udert, K.M.; Lienert, J. Nutrient cycles and resource management: Implications for the choice of wastewater treatment technology. *Water Sci. Technol.* **2007**, *56*, 229–237. [CrossRef]
12. KNBS. *2019 Kenya Population and Housing Census: Volume IV—Distibution of Population by Socio-Economic Characteristics*; KNBS: Nairobi, Kenya, 2019.
13. Kairigo, P.; Ngumba, E.; Sundberg, L.; Gachanja, A.; Tuhkanen, T. Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. *Sci. Total Environ.* **2020**, *720*, 137580. [CrossRef] [PubMed]
14. Ngumba, E.; Anthony, G.; Tuhkanen, T. Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Sci. Total Environ.* **2016**, *539*, 206–213. [CrossRef] [PubMed]
15. Subedi, B.; Balakrishna, K.; Joshua, D.I.; Kannan, K. Mass loading and removal of pharmaceuticals and personal care products including psychoactives, antihypertensives, and antibiotics in two sewage treatment plants in Southern India. *Chemosphere* **2017**, *167*, 429–437. [CrossRef] [PubMed]
16. Ngumba, E.; Kosunen, P.; Gachanja, A.; Tuhkanen, T. A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using spe-lc-ms/ms and matrix-matched standards. *Anal. Methods* **2016**, *8*, 6720–6729. [CrossRef]

17. Danner, M.-C.; Robertson, A.; Behrends, V.; Reiss, J. Antibiotic pollution in surface fresh waters: Occurrence and effects. *Sci. Total Environ.* **2019**, *664*, 793–804. [CrossRef] [PubMed]
18. Fatta-Kassinos, D.; Meric, S.; Nikolaou, A. Pharmaceutical residues in environmental waters and wastewater: Current state of knowledge and future research. *Anal. Bioanal. Chem.* **2011**, *399*, 251–275. [CrossRef]
19. Archer, E.; Petrie, B.; Kasprzyk-Hordern, B.; Wolfaardt, G.M. The fate of pharmaceuticals and personal care products (PPCPS), endocrine disrupting contaminants (EDCS), metabolites and illicit drugs in a wwtw and environmental waters. *Chemosphere* **2017**, *174*, 437–446. [CrossRef]
20. Lindberg, R.H.; Wennberg, P.; Johansson, M.I.; Tysklind, M.; Andersson, B.A.V. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environ. Sci. Technol.* **2005**, *39*, 3421–3429. [CrossRef]
21. Ignatev, A.; Tuhkanen, T. Monitoring WWTP performance using size-exclusion chromatography with simultaneous uv and fluorescence detection to track recalcitrant wastewater fractions. *Chemosphere* **2019**, *214*, 587–597. [CrossRef]
22. Michael, I.; Rizzo, L.; McArdell, C.S.; Manaia, C.M.; Merlin, C.; Schwartz, T.; Dagot, C.; Fatta-Kassinos, D. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Res.* **2013**, *47*, 957–995. [CrossRef]
23. Kümmerer, K. Antibiotics in the aquatic environment—A review—Part II. *Chemosphere* **2009**, *75*, 435–441. [CrossRef] [PubMed]
24. World Health Organization. WHO|HIV/AIDS. Available online: https://www.who.int/gho/hiv/en/ (accessed on 22 March 2019).
25. World Health Organization. WHO|Antiretroviral Therapy (ART) Coverage among All Age Groups. Available online: https://www.who.int/gho/hiv/epidemic_response/ART/en/ (accessed on 22 March 2019).
26. Swanepoel, C.; Bouwman, H.; Pieters, R.; Bezuidenhout, C. *Presence, Concentrations and Potential Implications of Hiv-Anti-Retrovirals in Selected Water Resources in South Africa*; Water Research Commission: Pretoria, South Africa, 2015.
27. Wood, T.P.; Duvenage, C.S.J.; Rohwer, E. The occurrence of anti-retroviral compounds used for HIV treatment in south african surface water. *Environ. Pollut.* **2015**, *199*, 235–243. [CrossRef] [PubMed]
28. Ncube, S.; Madikizela, L.M.; Chimuka, L.; Nindi, M.M. Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. *Water Res.* **2018**, *145*, 231–247. [CrossRef] [PubMed]
29. K'oreje, K.O.; Vergeynst, L.; Ombaka, D.; De Wispelaere, P.; Okoth, M.; Van Langenhove, H.; Demeestere, K. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu City, Kenya. *Chemosphere* **2016**, *149*, 238–244. [CrossRef] [PubMed]
30. Kandie, F.J.; Krauss, M.; Beckers, L.M.; Massei, R.; Fillinger, U.; Becker, J.; Liess, M.; Torto, B.; Brack, W. Occurrence and risk assessment of organic micropollutants in freshwater systems within the Lake Victoria South Basin, Kenya. *Sci. Total Environ.* **2020**, *714*, 136748. [CrossRef] [PubMed]
31. Segura, P.A.; Takada, H.; Correa, J.A.; El Saadi, K.; Koike, T.; Onwona-Agyeman, S.; Ofosu-Anim, J.; Sabi, E.B.; Wasonga, O.V.; Mghalu, J.M.; et al. Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries. *Environ. Int.* **2015**, *80*, 89–97. [CrossRef]
32. Bagnis, S.; Boxall, A.; Gachanja, A.; Fitzsimons, M.; Murigi, M.; Snape, J.; Tappin, A.; Wilkinson, J.; Comber, S. Characterization of the Nairobi River catchment impact zone and occurrence of pharmaceuticals: Implications for an impact zone inclusive environmental risk assessment. *Sci. Total Environ.* **2020**, *703*. [CrossRef] [PubMed]
33. Abou-Elwafa Abdallah, M.; Nguyen, K.H.; Ebele, A.J.; Atia, N.N.; Ali, H.R.H.; Harrad, S. A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry. *J. Chromatogr. A* **2019**, *1588*, 68–76. [CrossRef]
34. Oluwatosin, O.; Adekunle, B.; Obih, U.; Arne, H. Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. *J. Environ. Chem. Ecotoxicol.* **2016**, *8*, 14–24. [CrossRef]
35. Madikizela, L.M.; Ncube, S.; Chimuka, L. Analysis, Occurrence and removal of pharmaceuticals in african water resources: A current status. *J. Environ. Manag.* **2020**, *253*, 109741. [CrossRef]

36. Nannou, C.; Ofrydopoulou, A.; Evgenidou, E.; Heath, D.; Heath, E.; Lambropoulou, D. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. *Sci. Total Environ.* **2020**, 134322. [CrossRef] [PubMed]
37. Sabri, N.A.; Schmitt, H.; Van der Zaan, B.; Gerritsen, H.W.; Zuidema, T.; Rijnaarts, H.H.M.; Langenhoff, A.A.M. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. *J. Environ. Chem. Eng.* **2018**, 102245. [CrossRef]
38. Vilchèze, C.; Jacobs, W.R. The combination of sulfamethoxazole, trimethoprim, and isoniazid or rifampin is bactericidal and prevents the emergence of drug resistance in mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **2012**, *56*, 5142–5148. [CrossRef] [PubMed]
39. Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A.K.M.; Wertheim, H.F.L.; Sumpradit, N.; Vlieghe, E.; Hara, G.L.; Gould, I.M.; Goossens, H.; et al. Antibiotic resistance-the need for global solutions. *Lancet Infect. Dis.* **2013**, 1057–1098. [CrossRef]
40. CDC. *Antibiotic Resistance Threats in the United States*; Center for Disease Control and Prevention: Atlanta, GA, USA, 2019. [CrossRef]
41. WHO. *Global Tuberculosis Report 2017*; WHO: Geneva, Switzerland, 2017.

III

PRESENCE OF ANTIBIOTICS AND ANTIBIOTIC RESISTANT GENES IN URBAN HYDROLOGICAL CYCLES OF LUSAKA, ZAMBIA USING HIGH-THROUGHPUT QUANTITATIVE PCR ANALYSIS

by

Pius Kairigo, Daniel de Oliveira Patricio, Pinja Laukkanen, Elijah Ngumba, James Nyirenda, Nicholas Kasoma, Anthony Gachanja, Tuula Tuhkanen & Lotta-Riina Sundberg 2024

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IV

REMOVAL OF SELECTED ANTIBIOTICS AND ANTIRETROVIRAL DRUGS FROM HYDROLYZED URINE USING POWDERED ACTIVATED CARBON

by

Pius Kairigo, Johannes Ilomäki, Elijah Ngumba, Josephine Ouma, Edwin Madivoli, Austine Otieno, Anthony Gachanja & Tuula Tuhkanen
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