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Occurrence and resistance risk assessment of selected antimicrobials in surface waters of Juja, Kenya

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This thesis aimed to investigate the occurrence of selected antibiotics and antiretrovirals in the surface water of Juja, Kenya. Juja is a fast-growing town near Nairobi, which can be described by inadequate sanitation and relatively high usage of antibiotics and antiretrovirals for the treatment of HIV/AIDS as well as other infections. When entering the environment, these compounds can have adverse effects and exacerbate the problem of antibiotic resistance. The selected compounds included amoxicillin (AMX), ampicillin (AMP), tetracycline (TET), oxytetracycline (OXT), doxycycline (DOX), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfamethazine (SMZ), trimethoprim (TMP), carbamazepine (CBZ), lamivudine (3TC), and nevirapine (NVP). The occurrence of these was investigated by collecting water samples from the unlined open drains, a river, and a pond in the study area. Sampling was conducted in two rounds. Samples were extracted using solid-phase extraction and then analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Based on the measured concentrations, the risk for resistance development was evaluated by calculating risk quotient (RQ) values. Most of the studied compounds were detected in the samples, except for OXT and SDZ. Overall, the concentration ranged from 17 ng/l to 4269 ng/l. In both sampling rounds, SMX was detected with the highest concentration of antibiotics, while 3TC was the most abundant antiretroviral. The concentrations during the second sampling were significantly higher compared to the first sampling, with the total sum of concentrations being almost two times higher. The concentration between sampling points varied, with the highest concentration measured from open drains in more densely populated areas and close to the old WWTP. Measured concentrations in a river and a pond were generally lower compared to open drains. Differences in concentrations can be influenced by varying environmental conditions as well as different consumption levels and the physicochemical properties of the compounds. The measured concentrations in this study were lower compared to previous studies conducted in the same region and other sub-Saharan countries. However, the order of the compounds and the most detected compounds were similar. When compared to high-income countries, the concentrations were higher. The RQs ranged between 0.01 and 1.01, which indicates a low to high risk for the development of antibiotic resistance. TMP posed the highest risk for resistance selection. Further research on the subject, particularly regarding the development and occurrence of antibiotic resistance, is needed.

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Tämän tutkimuksen tavoitteena oli määrittää valittujen antibioottien ja antiretroviraalien esiintymistä Jujan pintavesissä. Juja on suhteellisen suuri kaupunki Keniassa, Nairobin lähellä. Alueella veden käsittely on puutteellista ja antibioottien sekä antiretroviraalien käyttö korkeaa. Ympäristössä näillä yhdisteillä voi olla haitallisia vaikutuksia ja ne voivat pahentaa antibioottiresistenssi ongelmaa. Tutkitut lääkeaineet olivat amoksisilliini (AMX), ampisilliini (AMP), tetrasykliini (TET), oksitetrasykliini (OXT), doksisykliini (DOX), sulfametoksatsoli (SMX), sulfadiatsiini (SZD), sulfametatsiini (SMZ), trimeropriimi (TMP), karbamatsepiini (CBZ), lamivudiini (3TC) ja nevirapiini (NVP). Valittujen lääkeaineiden esiintymistä tutkittiin keräämällä vesinäytteitä avoimista viemäriojista, joesta sekä alueella olevasta lammesta. Näytteenotto suoritettiin kahdessa erässä. Näytteet analysoitiin nestekromatografia-tandem-massaspektrometrillä (LS-MS/MS), jota ennen ne esikäsiteltiin kiinteäfaasiuutolla. Havaittujen pitoisuuksien perusteella myös riski antibioottiresistenssin kehittymiselle arvioitiin laskemalla riskiosamäärä (RQ) arvot. Lähes kaikki tutkitut yhdisteet havaittiin näytteistä, oksitetrasykliiniä ja sulfadiatsiinia lukuun ottamatta. Yhdisteiden pitoisuus vaihtele välillä 17 ng/l – 4269 ng/l. Molemmilla näytteenottokierroksilla SMX oli eniten havaittu antibiootti, kun taas 3TC oli runsain antiretroviraali. Yhdisteiden pitoisuudet olivat huomattavasti korkeampia toisen näytteenoton aikana, joka voi johtua esimerkiksi sademäärien ja olosuhteiden muutoksista. Myös näytteenottopaikkojen välillä havaittiin eroja. Korkeimmat pitoisuudet havaittiin tiheämmin asutuilta aluilta sekä läheltä vanhaa jätevedenpuhdistamoa, joissa kuormitus ympäristöön oli todennäköisesti korkeampaa. Joesta mitatut pitoisuudet olivat matalampia, mikä saattoi johtua yhdisteiden suuremmasta laimenemisesta veteen. Tutkimuksessa mitatut pitoisuudet olivat yleisesti alhaisempia kuin alueella ja Keniassa aikaisemmin tehdyssä tutkimuksissa. Pitoisuudet olivat kuitenkin korkeampia moniin korkean tulotason maihin verrattuna. Antibioottiresistenssin kehittymisriski vaihteli yhdisteiden välillä matalasta korkeaan. Suurin riski resistenssin kehittymiselle arvioitiin olevan trimetopriimillä (RQ = 1,01). Lisätutkimusta aiheesta, sekä erityisesti antibioottiresistenssin kehittymisestä ja esiintymisestä, tarvitaan.

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ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired immune deficiency syndrome
ARB	Antibiotic resistance bacteria
ARG	Antibiotic resistance genes
ART	Antiretroviral therapy
ARVD	Antiretroviral drug
AMX	Amoxicillin
AMP	Ampicillin
CBZ	Carbamazepine
DOX	Doxycycline
HIV	Human immunodeficiency virus
K_d	Sorption coefficient
K_{ow}	Octanol-water partition coefficient
LC-MS/MS	Liquid-chromatography-tandem-mass-spectrometry
LMIC	Low- and medium-income country
LOD	Limit of detection
LOQ	Limit of quantification
MEC	Measured environmental concentration
NVP	Nevirapine
OXT	Oxytetracycline
PNEC	Predicted no-effect concentration
RQ	Risk quotient
SDZ	Sulfadiazine
SMX	Sulfamethoxazole
SMZ	Sulfamethazine
SPE	Solid-phase extraction
TET	Tetracycline
TMP	Trimethoprim
WWTP	Wastewater treatment plant

1 INTRODUCTION

Pharmaceuticals have been receiving increasing attention in recent decades due to their role as emerging environmental pollutants (Cismaz et al. 2015, K'oreje et al. 2020). Antimicrobials, such as antibiotics and antiretrovirals, are a major group of pharmaceuticals that are widely used to prevent and treat various infections (Martino 2022). The development and use of antibiotics have led to declining mortality and disease rates, and their discovery is considered one of the most significant cornerstones in medical history (Carvalho & Santos 2016). The consumption of antibiotics has significantly increased in recent decades due to a growing population and investments in the health sector (aus der Beek et al. 2016, Klein et al. 2018, Browne et al. 2021). Consumption has increased especially in low- and middle-income countries (LMICs) where growing GDPs, rising incomes, urbanization, and ease in accessibility have accelerated consumption. From 2000 to 2015, the use has increased 65 % in defined daily doses (DDD), and by 2030 the consumption is predicted to increase by 200 %. (Klein et al. 2018) The extensive usage of antibiotics has resulted in their ongoing release into the environment, leading to increasing concentrations of antibiotic compounds being detected (Kümmerer 2009). Residuals of antibiotics and pharmaceuticals have been found in soil, sediments, and both ground and surface waters worldwide (aus der Beek et al. 2016). This has attracted significant attention due to its potential harm to humans and ecosystems (Heberer 2002, Cismaz et al. 2015, Gothwal & Shashidhar 2015, Yang et al. 2021). Particularly concerning is the increasing occurrence of antibiotic resistance bacteria, which can jeopardize the effectiveness of antibiotics and pose a major global healthcare problem (Ventola et al. 2015). The problem is global but especially concerning and challenging in many developing countries (Segura et al. 2015, Agunbiade & Moodley 2016). The aim of this thesis is to investigate the presence of specific antimicrobials in surface water in Juja, Kenya. The following chapters will briefly discuss topics related to antimicrobials, their pathways and sources in the environment, their potential effects, and the development of antibiotic resistance. The presence of antibiotics in the environment and the situation in developing countries, particularly in Kenya, will also be addressed. At the end of the introduction, the purpose and objectives of the thesis will be discussed.

1.1 Antimicrobials

Antimicrobials are a wide group of pharmaceuticals that are used to prevent and treat a range of infections. They target the specific components of the microorganisms to either kill them or prevent their growth or replication. (Madigan et al. 2019, Martino et al. 2020) Two main classes of antimicrobials discussed in this study are antibiotics and antiretrovirals. Antibiotics are especially used for the prevention and treatment of bacterial infections (Gothwal

& Shashidhar 2014). Their use in agriculture, for example as growth promoters, is also common (Van Boeckel et al. 2015). Antibiotics can be classified for example based on their structure, action mechanism, or route of administration. Examples of antibiotic classes based on their mechanism of action include beta-lactams, sulfonamides, aminoglycosides, glycopeptides, lincosamides, macrolides, rifamycins, tetracyclines, chloramphenicol, quinolones, and fluoroquinolones. (Gothwal & Shashidhar 2014) Some main classes of antibiotics investigated in this thesis include sulfonamides, tetracyclines, and beta-lactams, all of which are widely used for multiple purposes. Sulfonamides are one of the oldest class of antibiotics and they function by disrupting the synthesis of folic acid within bacteria cells. Folic acid is an essential nutrient for bacteria. Some main sulfonamides, studied in also this study, include sulfamethoxazole (SMX), sulfamethazine (SMZ), and sulfadiazine (SDZ). (Nunes et al. 2020) Tetracyclines function by inhibiting the protein synthesis of bacteria. There are diverse groups of tetracyclines, some naturally produced and some synthetic. (Nguyen et al. 2014) Tetracyclines investigated in this study include tetracycline (TET), doxycycline (DOX), and oxytetracycline (OXT). Beta-lactam antibiotics work by targeting the cell wall of bacteria by binding to the specific enzymes called penicillin-binding proteins (PBPs) that are important in building and maintaining the cell wall of bacteria. They are easily reactive and degradative due to the beta-lactam ring in their structure. (Fernandes et al. 2013) Selected beta-lactams in this study included ampicillin (AMP) and amoxicillin (AMX). In addition, also trimethoprim (TMP) and carbamazepine (CBZ), were investigated in this study. Trimethoprim functions by targeting the DNA synthesis of bacteria and it is often prescribed together with sulfamethoxazole. Carbamazepine is an anticonvulsant drug mainly used in the treatment of epilepsy (Drugbank 2024). Each class of antibiotics has varying physicochemical properties which influence their behavior (Ozumchelouei et al. 2019, Harrower et al 2021). The physicochemical properties of antibiotics will be discussed later.

Antiretroviral drugs are specifically used in the treatment of viral infections. Antiretroviral drugs can have various mechanisms of action. They can either target the viral functions, such as proteases, or the cellular functions that the virus needs. (Kausar et al. 2021) Unlike antibiotics, antiretrovirals are not extensively used for agricultural purposes (Jain et al. 2013). Antiretroviral (ARV) drugs are commonly utilized in the treatment of human immunodeficiency virus (HIV). ARV drugs used in the treatment of HIV can be classified into three categories: primary reverse-transcriptase inhibitors (NRTIs) and nucleotide reverse-transcriptase inhibitors (NtRTIs), secondary NRTIs, and non-nucleoside reverse-transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INSTIs). In this thesis selected antiretroviral drugs investigated include lamivudine (3TC), which is secondary NRTIs, and nevirapine (NVP), which is NNRTIs. (WHO 2021) Both of them are widely used to treat HIV infection. Additionally, lamivudine is also used in the treatment of hepatitis B infections. (Drugbank 2024)

1.2 Sources and pathways to the environment

Antibiotics and other pharmaceuticals can enter the environment through various pathways (Kümmerer 2009, Gothwal & Shashidhar 2015). The main source of antibiotics in the environment is the release of treated and untreated wastewater and excreta (Kümmerer 2009, Ngumba et al. 2016a, Archundia et al. 2017). Antibiotic compounds typically enter wastewater through urine and feces after consumption. These compounds are often only partially metabolized, leading to a significant amount of consumed antibiotics being excreted through urine and feces. (Zhou et al. 2021) Compounds can be excreted in their original form or as metabolites (Yang et al. 2021). Therefore, it is not only important to monitor parent compounds in the environment but also their metabolites (Felis et al. 2020). In industrialized countries, where wastewater and excreta treatment are usually effective, wastewater treatment plants (WWTPs) are the primary sources of antibiotics released into the environment (Michael et al. 2013). The highest concentrations of antibiotics are usually found in wastewater from hospitals and other medical clinics (Szymanska et al. 2019). Conventional WWTPs are not typically designed to effectively remove small micropollutants, such as pharmaceutical ingredients, allowing residual antibiotics to enter the environment with wastewater (Vieno et al. 2006, Michael et al. 2013). The removal of pharmaceutical and antibiotic compounds in WWTPs has been extensively studied. Removal efficiencies vary a lot depending on the compound properties and treatment technology used, but most WWTPs have low efficiency in removing residual antibiotics (Verlicchi et al. 2012, Adekoge et al. 2018). For example, beta-lactam antibiotics, such as penicillin, are usually relatively biodegradable in WWTPs (Felis et al. 2020). According to research, the most effective treatment methods include oxidation, reverse osmosis, and sorption on activated carbon (Szymanska et al. 2019). The situation is particularly concerning in many developing countries, where centralized systems serve only a fraction of the population. In these regions, the uncontrolled release of untreated wastewater and excreta into the environment are significant sources of contamination. (Wang et al. 2014, K'oreje et al. 2020)

In addition to untreated and treated wastewater, there are also other sources of antibiotic compounds in the environment. Antibiotics and antimicrobials are commonly used in agriculture and aquaculture for veterinary purposes (Van Boeckel et al. 2015, Kovalakova et al. 2020). Pharmaceuticals used on animals in pastures or through animal manure as fertilizer can release these compounds into the environment (Boxall et al. 2002, Kümmerer 2009). Also, the use of sludge from wastewater treatment processes, for example as fertilizer on agricultural lands, can introduce antibiotic compounds into the soil. Sludge is produced during the biological treatment of wastewater in which pharmaceutical compounds can absorb the sludge or biosolids. (Michael et al. 2013) Different compounds have varying rates of absorption, with some antibiotics, like tetracyclines, being absorbed more efficiently than others (Yang et al. 2021). The extent to which compounds are absorbed into the sludge and solid matter depends on various factors, including the properties of the pharmaceuticals, the properties of the wastewater, and the treatment process (Verlicchi et al. 2012).

Compounds with higher hydrophobicity and a higher octanol-water partition coefficient (K_{ow}) are more likely to be absorbed into the sludge, while compounds that are more hydrophilic and have a lower molecular weight remain in the water phase. The K_{ow} value is commonly used to estimate the tendency to accumulate in sludge (Michael et al. 2013). The sorption coefficient (K_d) is also used to represent the ratio between the concentration of the compound in the water phase and in sludge or organic matter in general. Higher K_d values indicate a greater sorption of the compound to the sludge. (Seifrtová et al. 2009, Michael et al. 2013) Antibiotics and other pharmaceutical compounds from agricultural lands and soil can end up in surface or groundwater through runoff (Boxall et al. 2002, Puckowski et al. 2016). It has been predicted that global antimicrobial consumption in agriculture will increase by 67% between 2010 and 2030, leading to an even higher environmental burden of antibiotic compounds (Van Boeckel et al. 2015). Especially the use in aquaculture has been increasing rapidly (Harrower et al. 2021).

Disposal of solid pharmaceutical and antibiotic waste is also one of the sources (Gothwal & Shashidhar 2015). Especially in developing countries, the incorrect disposal of unused and expired antibiotics and pharmaceuticals is common (Gitaka et al. 2020). From landfills and soil, compounds can be carried by runoff into the surface and ground waters (Adekoge et al. 2018, Harrower et al. 2021). Antibiotic compounds can also end up in drinking water and in the food chain, which poses a risk of human exposure (Yang et al. 2021). Figure 1 summarizes and presents the major sources and pathways for pharmaceuticals to the environment.

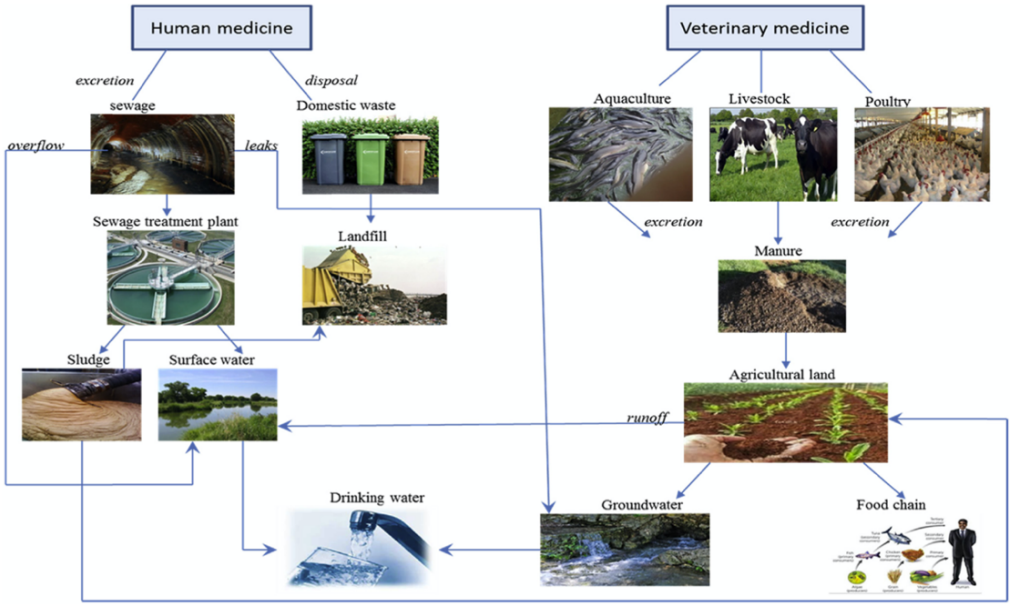


Figure 1. The main sources and pathways of pharmaceuticals in the environment. (Ebele et al. 2017, CC BY-NC-ND license.)

After being released into the environment, the fate and function of the compounds are affected by their physicochemical properties and the conditions of the environment (Kümmerer 2009). Pharmaceuticals can be partitioned into various environmental compartments based on their physicochemical properties (Harrower et al. 2021). Important physicochemical parameters are for example water solubility (S), octanol-water partition coefficient (K_{ow}), distribution coefficient (K_d), and acid dissociation constant (pK_a) (Ozumchelouei et al. 2020). Physicochemical parameters vary a lot between different compounds. For example, sulfonamides have usually relatively low K_{ow} values, meaning they are more likely to stay in aqueous compartments. K_{ow} value cannot be alone used to predict the absorption. For example, tetracyclines have typically low K_{ow} values, but due to their high K_d values and numerous functional groups, they often bind to solid matter. (Harrower et al. 2021) Environmental conditions, such as pH, climate conditions, soil type, and a variety of other factors can also affect the fate of compounds (Puckowski et al. 2016, Harrower et al. 2021). For example, the sorption of sulfamethazine has been shown to increase in lower pH compared to higher pH (Lertpaitoonpan et al. 2009). In a study by Liu et al. (2020), pH also affected the sorption of oxytetracycline onto organic matter. Temperature can also influence a lot the persistence in the soil. For example, oxytetracycline has been shown to be more persistent in low temperatures. (Harrower et al. 2021) Understanding the physicochemical properties of the compounds and the influence of environmental conditions is important in predicting the behavior of antibiotic compounds on the environment.

1.3 Effects in the environment

In the environment, antibiotics and other antimicrobials can have various impacts. They are considered to be persistent or pseudo-persistent compounds as they enter the environment more rapidly than they are eliminated. (Gothwal & Shashidhar 2015). Typically, the amount of these compounds entering the environment is relatively low, but due to continuous release and their persistence, antibiotic compounds can have long-term and adverse effects on non-target organisms (Puckowski et al. 2016). Ecotoxicological risks and effects have been studied in numerous studies and it has been shown that antibiotic compounds can have toxic effects for example on micro-organisms, plants, and animals. (Kümmerer 2009, Cizmas et al. 2015, Yang et al. 2021) The toxicity and effects depend on the compound and the organisms being studied (Yang et al. 2021).

Effects have been studied both in terrestrial and aquatic environments. It has been shown that antibiotics can disturb basic environmental functions, such as microbial activities and nutrient cycles (Adekoge et al. 2018, Kovalakova et al. 2020). It has also been found that primary producer appears to be particularly vulnerable to the adverse effects of antibiotics (Kovalakova et al. 2020). Plants and vegetation can absorb antibiotics, resulting in antibiotic residues being discovered in crop plants in many places. (Puckowski et al. 2016, Adekoge et al. 2018) Antibiotic compounds have been shown to have negative effects on plant growth and functions, for example by inhibiting seed germination and root elongation, and altering malondialdehyde (MDA) contents and antioxidative

enzyme activities (Yang et al. 2021). Antibiotic compounds can bioaccumulate in the food chains, eventually reaching higher organisms (Puckowski et al. 2016). In aquatic environments, the effects of antibiotics on fish have been investigated, revealing physiological changes, impacts on nervous systems, and toxic effects on reproductivity (Yang et al. 2021). Despite extensive research on the topic, there is a need for more research on the trophic transfer and toxicity to biota (Puckowski et al. 2016). Most studies have focused on the effects of individual antibiotics, while the combined effects of different antibiotic compounds are less studied (Kairigo et al. 2020b, Yang et al. 2021) The effects of the metabolites and transformation products of parent compounds are also less studied. The transformation products have been shown to potentially be more toxic than the parent compounds. (Archundia et al. 2017) Furthermore, the effects on humans consuming organisms exposed to antibiotic compounds require further investigation (Adekoge et al. 2018).

1.4 Antimicrobial resistance

One of the most concerning issues related to residual antibiotics in the environment is the development of antibiotic resistance. According to the World Health Organization (WHO), antimicrobial resistance poses a significant risk to both global health and human development (WHO 2023). Antimicrobial resistance endangers the effectiveness of antimicrobials, which can have serious effects on public health. It has been estimated that 700 000 deaths are caused annually by antimicrobial resistance worldwide, and if the problem is neglected, 10 million people could die due to the problem by 2050. (O'Flaherty & Cummins 2017) Antibiotic resistance also impacts the economy by increasing healthcare costs and reducing labor supply and efficiency (Kariuki et al. 2022). The development of antibiotic resistance can occur naturally, but it has been shown that there is a strong association between antibiotic resistance and levels of antibiotic consumption (Larsson & Flach 2022). In many studies, human activities, especially the overuse and misuse of antibiotics, are named as the main cause for the enrichment and spread of antibiotic resistance (for example Gullberg et al. 2011, Rizzo et al. 2013, O'Flaherty et al. 2017, Yang et al. 2021).

The formation of antibiotic resistance genes can occur in various ways (Alanis 2005, Amarasiri et al. 2019). Antibiotics target bacteria, affecting their growth and survival by inhibiting or killing them, without causing harm to the host. Most antibiotics target either the bacterial cell membrane or cell wall structure or essential molecular processes needed for bacterial growth and survival (Madigan et al. 2019). Antibiotic resistance refers to bacteria's capacity to withstand the effects of antibiotics (Amarasiri et al 2019). Resistance can occur through mutations or by acquiring antibiotic resistance genes (ARGs) from other bacteria or the environment (Alanis 2005, Amarasiri et al. 2019). Mutations occur in DNA during replication, and mutant strains can transfer the mutation to their progeny through vertical transfer (Mancuso et al. 2021). Bacteria can obtain antibiotic resistance genes also through horizontal gene transfer (HGT), which can occur through transformation, transduction, or conjugation. This transfer is often facilitated by mobile genetic elements (MGEs), such as plasmids or

transposons, which can be easily transferred between bacteria of the same or different species through horizontal gene flow. (Amarasiri et al. 2019, Madigan et al. 2019) Bacteria can also incorporate various resistance genes, leading to the development of multi-resistant bacteria. These bacteria exhibit high resistance to multiple antibiotics, making their treatment more challenging and costly. (Amarasiri et al. 2019)

Development of resistance can occur naturally, but research has shown that antibiotic residuals in the environment can drive the formation. Increasing loads of residual antibiotics have exacerbated the problem. (Harrower et al. 2021) The discharge of residual antibiotics into the environment exposes micro-organisms to sub-lethal doses of antibiotics creating a selective pressure that can favor bacteria with resistant strains. (Adekoge et al. 2018) It has been shown for several antibiotics, that even extremely low concentrations, below the minimum inhibitory concentration (MIC), can promote the development of antibiotic resistance (Gullberg et al. 2011, Adekoge et al. 2018). Lower concentrations provide a competitive advantage for the proliferation of resistant strains (Andersson & Hughes 2014, Khan et al. 2017, Amarasiri et al. 2019) and are sufficient to sustain the survival of resistant bacteria in the population (Gullberg et al. 2011).

The environment has been recognized as a crucial factor in the development and dissemination of antibiotic resistance genes and bacteria. The environment is a natural source and reservoir of resistance genes, but it also receives antibiotic resistance genes (ARGs) and bacteria (ARB) from anthropogenic sources (Rizzo et al. 2013, Manaia et al. 2016, Amarasiri et al. 2019). Effluents from WWTPs and untreated wastewater are reported as the primary sources for spreading resistance genes and bacteria into the environment (Rizzo et al. 2013, Karkman et al. 2018). Various ARGs have been found in all kinds of wastewater worldwide (Zhang et al. 2009, Pazda et al. 2019). WWTPs receive ARGs and ARBs from various sources, and the treatment processes can also create a suitable environment for the development of antibiotic resistance genes and bacteria. For example, during the biological treatment process, bacteria are continuously mixed and exposed to low levels of antibiotics in the wastewater, which can lead to the formation of resistance. (Rizzo et al. 2012, Michael et al. 2013, Manaia et al. 2016, O'Flaherty & Cummins 2017) It has also shown that some disinfection by-products and metals can influence to formation of ARGs and ARBs. For instance, chlorination in treatment plants or the presence of metal ions in water can increase the formation of antibiotic resistance. (Amarasiri et al. 2019)

When wastewater is discharged, ARGs and ARBs enter the environment. Various environmental factors can affect the selection of resistance genes and bacteria in the environment (Manaia et al. 2016). ARGs and ARBs have been widely detected in biota, soil, sediments, surface, and ground waters (Yang et al. 2021). ARGs and ARBs can also be transmitted to humans for example by utilizing contaminated water for irrigation or as a source of drinking water or through food chains (O'Flaherty & Cummins 2017, Adekoge et al. 2018, Amarasiri et al. 2019). The potential human health risks have not been fully evaluated, and further research is needed to understand the factors influencing the expression and spread of resistance genes (Bengtsson-Palme et al. 2018,

Amarasiri et al. 2019). However, the risk for antibiotic resistance development can be estimated. One approach to estimate the potential risk is to derivate the risk quotient (RQ) value. It is calculated by comparing predicted no-effect concentration (PNEC) to measured environmental concentration (MEC) (Hernando et al. 2006) It is more often used for evaluating the potential ecological risk of compounds but is also suitable for resistance risk assessment. For example, Bengtsson-Palme & Larsson (2016) have estimated the PNEC values for resistance selection for numerous antibiotics and antibiotic combinations. Similarly to antibiotic resistance, viruses can also develop resistance against antiretrovirals. Research of antiretroviral resistance is scarcer compared to antibiotic resistance and should be studied more. (Jain et al. 2013)

1.5 Occurrence of antimicrobials in the environment

The occurrence of antibiotics and other pharmaceuticals has been widely studied and monitored in the past decades. In numerous studies, antibiotic residues, and resistance genes as well as bacteria have been detected and reported in various kinds of environments around the world. The environmental compartments that have been studied include for instance influents and effluents from WWTPs, drinking water, surface and ground water, soil, and sediments. (Agunbiade & Moodley 2015, K'oreje et al. 2020, Yang et al. 2021) The environmental concentration of antibiotics has been reported in the concentration range of µg/l to ng/l. Concentrations vary greatly between different compounds and environmental comportments. (Yang et al. 2021) Research has tended to be more focused on developed countries, whereas fewer studies have been conducted in developing countries (aus der Beek et al. 2016, K'oreje et al. 2020, Browne et al. 2021). The lack of research in developing countries is particularly concerning, given that in these regions, the use of antibiotics and pharmaceuticals is often high due to a higher disease burden (Agunbiade & Moodley 2016, Gitaka et al. 2020). In developing countries, antibiotics are also more easily available compared to developed countries, due to less strict regulation and lack of surveillance (Alanis 2005, Agunbiade & Moodley 2016, Gitaka et al. 2020). Antibiotics are for instance easy to get without prescription and self-medication is common. It has been estimated that almost 50 % of antibiotics consumed are unnecessary. (Karimi et al. 2023a) Combined with inadequate wastewater management practices and systems, the pharmaceutical loads to the environment are often high. Reported concentrations are significantly higher in low- and medium-income countries compared to higher-income countries. (Agunbiade & Moodley 2015, Segura et al. 2015) LMICs are also more affected by the development of antibiotic resistance (Gitaka et al. 2020, Larsson & Flach 2022), due to generally worse medical, social, and economic conditions (Carvalho & Santos et al. 2016).

Especially in Africa, the situation is concerning due to the high prevalence of diseases (Kairigo et al. 2020a). Especially the high prevalence of HIV/AIDS is concerning. In Africa, 25.7 million people are living with HIV/AIDS, which covers over 70 % of all the HIV/AIDS cases in the world. Over half of those are on antiretroviral therapy (ART) (Kairigo et al. 2020b), which involves taking

various antiretroviral drugs (ARDVs). Antiretrovirals slow down the multiplication of HIV but do not kill the virus, thus ART medication is usually lifelong (Ncube et al. 2018). The South and East African countries are most affected by HIV/AIDS, whereas the northern and western parts are less influenced (Frankema et al. 2022). Few studies on the occurrence of antibiotics and antiretrovirals have been conducted in Africa, but the research remains scarce and limited compared to developed countries (K'oreje et al. 2016, aus der Beek et al. 2015, Hawash et al. 2023, Addis et al. 2024). Most of the studies from Africa are from South Africa (K'oreje et al. 2020, Addis et al. 2024). Based on the studies, reported concentrations in Africa are shown to be much higher than for example in Europe (Fedaku et al. 2019). The aim of this study is to investigate the occurrence of selected antibiotics and antiretrovirals in the surface water of Kenya. In Kenya, residuals of antibiotics and other pharmaceuticals have been detected in various environmental compartments in several studies (for example K'oroje et al. 2016, Ngumba et al. 2016a, Muriuki et al. 2020, Chemtai et al. 2023). Table 1 presents the reported maximum concentrations of selected antibiotics and antiretrovirals investigated in this study from surface waters in Kenya and other African countries. For comparison, the reported values from higher-income countries have also been included in the table.

TABLE 1. Reported maximum concentration of selected antibiotics and antiretroviral drugs. AMX = amoxicillin, AMP = ampicillin, TET = tetracycline, OXT = oxytetracycline, DOX = doxycycline, SMX = sulfamethoxazole, SDZ = sulfadiazine, SMZ = sulfamethazine, TMP = trimethoprim, CBZ = carbamazepine, 3TC = lamivudine, NVP = nevirapine, n.d = not detected.

Compound	Surface water (ng/l)	Country	Reference
AMX	3300	Kenya	Muriuki et al. 2020
	900	Kenya	Kairigo et al. 2020a
	2	Kenya	Chemtai et al. 2023
	3410	Zambia	Ngumba et al. 2020
	29	China	Li et al. 2018
	n.d	Finland	Ngumba et al. 2016b
AMP	240	Kenya	Ngigi et al. 2020
	0.2	Kenya	Chemtai et al. 2023
	18	China	Li et al. 2018
	26	Germany	Christian et al. 2003
TET	0.2	Kenya	Chemtai et al. 2023
	<120	Kenya	Ngigi et al. 2020
	434000	Kenya	Segura et al. 2015
	465000	Ghana	Segura et al. 2015
	4220	Zambia	Ngumba et al. 2020
	1290	South Africa	Addis et al. 2024
	54	China	Chen et al. 2014
	228	Spain	López-Serna et al. 2011
	n.d	Finland	Ngumba et al. 2016b
OXT	60	South Africa	Segura et al. 2015
	220	China	Chen et al. 2014

	37	Spain	López-Serna et al. 2011
DOX	300	Kenya	Kairigo et al. 2020a
	5	Kenya	Segura et al. 2015
	3260	Zambia	Ngumba et al. 2020
	10	Ghana	Segura et al. 2015
	112	China	Chen et al. 2014
	48	Spain	López-Serna et al. 2011
	n.d	Finland	Ngumba et al. 2016b
SMX	506000	Kenya	Muriuki et al. 2020
	56600	Kenya	Kairigo et al. 2020a
	97 000	Kenya	Kairigo et al. 2020b
	13000	Kenya	Ngumba et al. 2016a
	39000	Kenya	K'oreje et al. 2016
	274	Kenya	Chemtai et al. 2023
	6840	Kenya	Ngigi et al. 2020
	11800	Zambia	Ngumba et al. 2020
	9640	Ghana	Segura et al. 2015
	10568	South Africa	Segura et al. 2015
	53828	Mozambique	Segura el al. 2015
	258	Asia	aus der Beek et al. 2016
	68	Europa	aus der Beek et al. 2016
	25	Finland	Ngumba et al. 2016b
SDZ	840	Kenya	Ngigi et al. 2020
	113	China	Chen et al. 2014
	23	Spain	López-Serna et al. 2011
SMZ	630	Kenya	K'oreje et al. 2016
	24	Kenya	Chemtai et al. 2023
	389	China	Chen et al. 2014
	55	Spain	López-Serna et al. 2011
TMP	479000	Kenya	Muriuki et al. 2020
	200	Kenya	Kairigo et al. 2020a
	4400	Kenya	Kairigo et al. 2020b
	2650	Kenya	Ngumba et al. 2016a
	6950	Kenya	K'oreje et al. 2016
	67	Kenya	Chemtai et al. 2023
	3160	Kenya	Ngigi et al. 2020
	2410	Zambia	Ngumba et al. 2020
	5875	South Africa	Segura et al. 2015
	1374	Ghana	Segura et al. 2015
	6220	Mozambique	Segura et al. 2015
	128	Asia	aus der Beek et al. 2016
	30	Spain	López-Serna et al. 2011
	15	Finland	Ngumba et al. 2016b
CBZ	430	Kenya	K'oreje et al. 2016
	868	Africa	aus der Beek et al. 2016
	26	Asia	aus der Beek et al. 2016
	188	Europe	aus der Beek et al. 2016

3TC	913000	Kenya	Muriuki et al. 2020
	167000	Kenya	K'oreje et al. 2016
	228300	Kenya	Kairigo et al. 2020b
	5428	Kenya	Ngumba et al. 2016
	49700	Zambia	Ngumba et al. 2020
	4.1	France	Aminot et al. 2015
	12	Finland	Ngumba et al. 2016b
NVP	145000	Kenya	Muriuki et al. 2020
	2300	Kenya	Kairigo et al. 2020b
	4859	Kenya	Ngumba et al. 2016a
	5620	Kenya	K'oreje et al. 2016
	220	Zambia	Ngumba et al. 2020
	1.3	France	Aminot et al. 2015
	n.d	Finland	Ngumba et al. 2016b

1.6 Kenya as a research context

Higher concentrations reported from Kenya and other sub-Saharan countries (Table 1) are affected by numerous factors. In many LMICs, the consumption of antibiotics and ARVDs is often high due to the high disease burden. The leading causes of death in Kenya are HIV/AIDS, respiratory infections, and diarrheal diseases (WHO 2022). The HIV/AIDS epidemic has heavily impacted Kenya, with a significant population living with the disease. It has been estimated that in 2022, 1.4 million people in Kenya were living with HIV infection with most of them being on antiretroviral therapy treatment (UNAIDS 2022). Antiretroviral therapy involves taking a combination of different antiretroviral drugs and it is the main treatment method for HIV/AIDS. Common antiretrovirals used in Kenya are for example nevirapine (NVP), zidovudine (ZDV), and lamivudine (3TC). (WHO 2016) HIV carriers are prone to other infections, which is why they often also need medication for other diseases such as tuberculosis or malaria (Kunin et al. 1995, Ngumba et al. 2016a). Antiretroviral are often administered together with antibiotics to prevent different coinfections. One of the most common antibiotics prescribed is cotrimoxazole, which is a combination antibiotic of sulfamethoxazole and trimethoprim. (WHO 2014, Karimi et al. 2023a) Extensive use of pharmaceuticals is also affected by inadequate regulation, resulting in high rates of self-medication and use without a prescription (Karimi et al. 2023b). It has been estimated that in Kenya, 70 % of consumed antibiotics are used without prescription (Kariuki et al. 2011). The use of antibiotics, especially tetracyclines, for veterinary purposes, is also high (Kariuki et al. 2011), which increases the environmental loads of antibiotic compounds.

In addition to the high usage of pharmaceuticals, the wastewater treatment management and sanitation facilities are poor in many places in Kenya (Kariuki et al. 2011, K'oroje et al. 2016). The capacity of WWTPs is limited and WWTPs serve only a fraction of the urban population. The majority of the population lives in informal settlements with poor sanitation and no connection to the sewage systems. In Kenya, the sewage connectivity is only 17 % varying a lot between the main cities. (Kenya Ministry of Health 2014) Poor sewage connectivity leads

to uncontrolled discharge of untreated wastewater and excreta into the environment. This results in residual pharmaceuticals ending up in the environment more widely and from multiple sources. Population growth, urbanization, and expansion of informal settlements exacerbate the problem. (Karimi et al. 2023a) Residuals of antibiotics and other pharmaceuticals have been detected in various environmental compartments in several studies in Kenya (for example Ngumba et al. 2016a, K'oroje et al. 2016, Muriuki et al. 2020, Chemtai et al. 2023).

The antibiotic residues in the environment and the rate of antimicrobial resistance in Kenya are concerning and increasing (Gitaka et al. 2020, WHO 2022). More pathogens are gaining resistance against antibiotics which endanger their efficiency and poses a risk to human health. The increasing resistance to anti-HIV drugs is particularly worrying. (Kariuki et al. 2011) Residuals of antibiotics and ARGs in the environment can also endanger safe drinking water and food production. In addition, it can affect the reuse possibilities of effluents and sludge from treatment plants for example in irrigation and as fertilizer. (Jimenez et al. 2009) This is a growing concern, particularly given the rising demand for water resources and the scarcity of water in the country (K'oroje et al. 2016). The safe handling and reuse of wastewater and sludge is crucial, making it important to understand the potential risks associated with the presence of antimicrobials and antimicrobial resistance. Antibiotic residuals and emerge of resistance, also endanger the achievement of the United Nations' Sustainable Development Goals (SDGs), especially those related to clean water, zero hunger, health, and reducing inequalities (UN 2020, Gajdács et al. 2021). Thus, research on the topic is crucial and still needed.

1.7 Aim of the study

This thesis aimed to investigate the presence of certain antibiotics and antiretroviral compounds in the surface water of Juja, Kenya. These antibiotics and antiretrovirals are commonly used in the area for treating conditions such as HIV/AIDS and its coinfections. Additionally, the study evaluated the risk of antibiotic resistance development based on the measured environmental concentrations of these compounds. The presence of the compounds was determined by analyzing surface water samples using the SPE-LC-MS/MS technique. The goal of the thesis was to provide updated information on the situation and compare the results to previous studies conducted in the region and globally. The research questions of the study were the following:

1) What are the measured concentrations of selected antibiotics and antiretrovirals in surface water samples?
2) What are the risks related to antibiotic resistance selection based on the measured concentration of the compounds?
3) How do the results of this study compare to previous studies conducted in the region and to other reported values in the literature?

Hypotheses were that concentrations of selected and typically consumed antibiotics and antiretrovirals are expected to be relatively high, especially when compared to the concentrations reported from high-income countries. Concentrations are probably similar to those typically observed in the region. A second hypothesis is that high concentrations of compounds also increase the potential risk for resistance selection among the selected compounds.

2 MATERIALS AND METHODS

The study aimed to investigate the occurrence of ten antibiotics and two antiretrovirals in surface water in Juja, Kenya (Figure 2). The antibiotic and antiretroviral compounds studied included amoxicillin (AMX), ampicillin (AMP), tetracycline (TET), oxytetracycline (OXT), doxycycline (DOX), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfamethazine (SMZ), trimethoprim (TMP), carbamazepine (CBZ), lamivudine (3TC), and nevirapine (NVP). Surface water samples for the analysis were collected from the Thiririka River and unlined open drains in different parts of Juja and Jomo Kenyatta University of Agriculture and Technology (JKUAT) located in the town. The extraction of analytes from water samples was accomplished via solid-phase extraction (SPE) using hydrophilic-lipophilic balance (HLB) SPE cartridges. The method is suitable for targeted pharmaceuticals (Ngumba et al. 2016b). Following the extraction, the liquid chromatography-tandem-mass spectrometry (LS-MS/MS) technique was employed for the identification and quantification of the pharmaceutical compounds from the samples. All analyses were carried out in the laboratory of JKUAT. Based on the measured concentrations of the compounds (MEC), the risk quotients (RQs) for the development of antibiotic resistance selection were calculated for each compound using the predicted no-effect concentrations values for resistance selection ($PNEC_{(RS)}$) presented by Bengtsson-Palme and Larsson (2016).

Figure 2. Juja is located near the capital of Kenya, Nairobi. Google Maps 2024.

2.1 Sampling plan

2.1.1 Study area

The study was carried out in Juja town in Kenya. The town of Juja is located in Kiambu County about 30 km from Nairobi between Thika and Ruiru towns. In 2019 the population of Juja subcounty was 300 948 (KNBS 2019a). The area is one of the fastest-urbanizing areas in Kenya. Population in the area has been growing and is expected to continue to grow (KNBS 2019c, KNBS 2019d). Urbanization and growing population are affected by the near location to Nairobi, the Thika superhighway, and the presence of the Jomo Kenyatta University of Agriculture and Technology (JKUAT) located in the town. The study was focused on the area near JKUAT University. The two main rivers in the area are the Ndarugu River and the Thiririka River (Figure 3). The use of pharmaceuticals in the area is high, especially those used in the treatment of HIV/AIDS and infections related to it. Kiambu County is one of the 10 high HIV burden counties in Kenya. (Kenya Ministry of Health 2014) In many parts of the Juja, the sewer system coverage is inadequate, and the decentralized systems are poorly designed. Only 5.5 % of the households are connected to the primary sewer system and most of the households have septic tanks or pit latrines. (KNBS 2019b) Septic tank systems in the area are often ineffective and not so well-designed. There are slightly different areas in Juja. Gachororo is a more slum-like area while Muchatha and Greenfield are more modern areas that mainly use septic tank systems. (Muriuki et al. 2020) Inadequate treatment and sewer system coverage leads to wastewater and excreta being discharged directly into the open drains and environment without proper purification or treatment. The weather in Juja is relatively warm, with the mean yearly temperature recorded to be 18.8 °C. The yearly precipitation level amounts to 1014 mm, with the monthly average being 28 mm. The highest amounts of rainfall are typically observed during April and May, as well as during October and November. The driest months generally fall between July and September. (Climate Data 2024)

Figure 3. Map of the study area. Satellites.pro 2024, edited.

2.1.2 Sampling

Sampling for the study was carried out in two rounds. The first round of sampling took place in October, and the second round occurred in November 2023. The rains in the area had started when sampling was conducted. The surface water samples were collected from ten different sampling points. The exact coordinates and descriptions of the sampling points are provided in Table 2, whereas Figure 4 presents locations on the map. The exact coordinates of the sampling points were determined using a Garmix Etrex GPS device. In the first sampling, the samples were collected from Thririka River (sampling points 1–2) and various open drains in Juja town (sampling places 5–10). In the second sampling the samples were collected inside of JKUAT (sampling places 3–4) and from the same open drains (sampling places 5–10) as in the first sampling round. Figure 5 is a picture from one of the sampling points from the river (sampling point 1) and Figure 6 is a picture from one of the open drains studied (sampling point 6). Sampled open drains were selected so that they covered the study area and represented different types of areas. All the open drains were unlined earthen drains. Plastic bottles were used in the collection of water samples. In both sampling and from each sampling point duplicate samples were collected. The samples were kept in a cool box during sampling and transportation to the laboratory. Before extraction samples were stored in the refrigerator (+ 4 °C).

TABLE 2. Sampling points of the study with the coordinates and descriptions of the places based on field observations.

Sampling point	Coordinate	Description
1. Thririka River	S01°05′12.4″ E036°59′21.0″	Sample from Thiririka River. The sampling place was surrounded by fields and located near some factories.
2. Thririka River	S01°06′47.4″ E037°00′44.2″	The second sample from the Thiririka River. The sampling point was next to the Nairobi superhighway.
3. JKUAT drain	S01°05′38.1″ E037°00′41.6″	Open drain/water trench inside of JKUAT University.
4. JKUAT pond	S01°06′00.6″ E037°00′49.9″	Pond inside of JKUAT University. Near the main gate of JKUAT (gate A).
5. Open drain	S01°06′13.7″ E037°00′56.7″	Open drain in Muchatha, Juja. Next to the road entering/exiting JKUAT and near to the main gate of JKUAT (gate A).
6. Open drain	S01°06′01.4″ E037°00′46.2″	Open drain in Muchatha, Juja. Next to Muramati Road and near gate C of JKUAT.
7. Open drain	S01°05′36.7″ E037°01′11.6″	Open drain in Gachororo, Juja. Next to the Gachororo Road and

		near to the JKUAT dam/semi-lake.
8. Open drain	S01°05′44.5″ E037°01′23.0″	Open drain in Gachororo, Juja. Near to the old WWTP in the area.
9. Open drain	S01°06′07.2″ E037°01′23.2″	Open drain Juja, other side of the Nairobi highway than JKUAT. Located in a densely populated area.
10. Open drain	S01°06′06.6″ E037°01′37.7″	The same open drain as the previous one but further away. Less buildings and apartments.

Figure 4. Map of the sampling points (1–10) of the study. Made with Google Maps 2024.

Figures 5 and 6. The first picture is from the Thiririka River (sampling point 1) and the second one from one of the open drains studied (sampling point 6).

2.2 Materials

2.2.1 Selected antimicrobials

The antibiotic and antiretroviral compounds selected in this study included amoxicillin (AMX), ampicillin (AMP), tetracycline (TET), oxytetracycline (OXT), doxycycline (DOX), sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfamethazine (SMZ), trimethoprim (TMP), carbamazepine (CBZ), lamivudine (3TC), and nevirapine (NVP). The pharmaceutical compounds were selected due to their abundant usage and easy availability. The physicochemical properties affecting the fate and partitioning of the target compounds are presented in Table 3, whereas the structures of the compounds are presented in Figure 7. Water solubility, pK_a and log K_{ow} values of studied compounds vary a lot.

TABLE 3. Physicochemical properties of selected pharmaceutical compounds of the study. pK_a = dissociation constant, log K_{ow} = octanol-water coefficient and logP = partition coefficient.

Compound	Molecular Formula	CAS no.	Molecular weight (g/mol)	Water solubility (mg/L)	pK_a	log K_{ow} /logP
Amoxicillin (AMX)	$C_{16}H_{19}N_3O_5S$	26787–78–0	365.4	958[a]	2.6[a]	0.87[a]
Ampicillin (AMP)	$C_{16}H_{19}N_3O_4S$	69-53-4	349.4	10100[a]	2.65, 7.25[a]	1.35[a]
Tetracycline (TET)	$C_{22}H_{24}N_2O_8$	60–54–8	444.4	231[a]	3.30, 7.68[a]	-1.37[a]

Oxytetracycline (OXT)	$C_{22}H_{24}N_2O_9$	79–57–2	460.4	47[a]	9.5[a]	-0.90[a]
Doxycycline (DOX)	$C_{22}H_{24}N_2O_8$	564-25-0	444.4	630[b]	3.09[b]	-0.72[b]
Sulfamethoxazole (SMX)	$C_{10}H_{11}N_3O_3S$	723–46–6	253.28	610[a]	1.6, 5.7[a]	0.89[a]
Sulfadiazine (SDZ)	$C_{10}H_{10}N_4O_2S$	68–35–9	250.28	77[a]	6.36[a]	-0.09[a]
Sulfamethazine (SMZ)	$C_{12}H_{14}N_4O_2S$	57-68-1	278.33	1500[a]	2.65, 7.49[a]	0.14[a]
Trimethoprim (TMP)	$C_{14}H_{18}N_4O_3$	738–70–5	290.3	400[a]	7.12[a]	0.91[a]
Carbamazepine (CBZ)	$C_{15}H_{12}N_2O$	298-46-4	236.27	35.4[a]	15.96, -3.8[a]	2.45[a]
Lamivudine (3TC)	$C_8H_{11}N_3O_3S$	134678–17–4	229.26	70000[a]	4.3, 14.29[b]	-9.54[a]
Nevirapine (NVP)	$C_{15}H_{14}N_4O$	129618–40–2	266.30	0.7046[b]	2.8a	3.89[a]

[a] = PubChem, https://pubchem.ncbi.nlm.nih.gov (Accessed on 23.4.2024)
[b] = Drugbank, https://go.drugbank.com (Accessed on 23.4.2024)

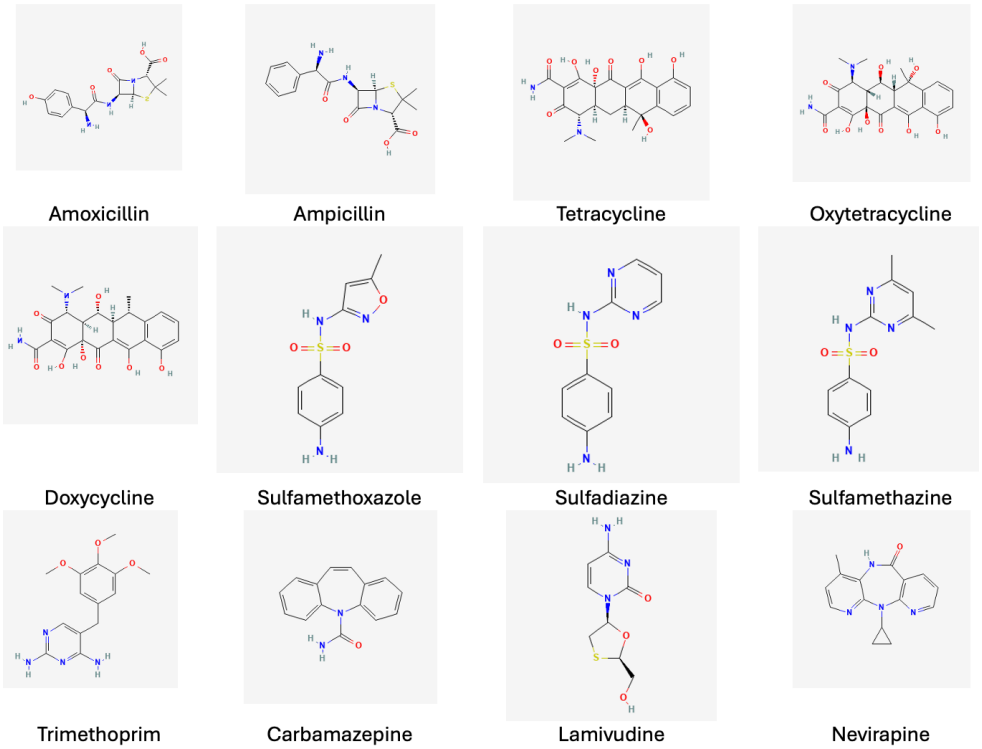


Figure 7. Molecular structures of studied pharmaceutical compound. Source: https://pubchem.ncbi.nlm.nih.gov (Accessed on 23.4.2024)

Analytical standards of the studied pharmaceuticals were obtained from Sigma Aldric (US) and Universal Pharmaceuticals Corporation (Kenya). The standards for sulfamethoxazole, amoxicillin, and trimethoprim were from Sigma

Aldric and the rest from the Universal Pharmaceuticals Corporation. All pharmaceutical standards were purity of >99%.

2.2.2 Stock solutions

Individual standards of each analyte were dissolved in methanol to a concentration of 1000 mg/l. From the individual standard solutions, the intermediate mixed standards (0.02 mg/ml) were prepared. A stock solution containing all the pharmaceuticals was prepared of the intermediate mixed standard solutions in methanol. Working standards in the range of 5–400 µg/l were prepared by appropriate dilutions in methanol: water (1:9) dilution of the stock solution.

2.2.3 Other chemicals and reagents

Glass microfiber filters 47 mm GF/D (2.7 µm) and GF/F (0.7 µm) for filtering the surface water samples were obtained from Whatman (Maidstone, England). Oasis Hydrophobic-Lipophilic Balance (HLB; 3 cc; 60 mg) solid-phase extraction (SPE) cartridges for the compound extraction were obtained from Waters Corporation (Milford, USA). Ultrapure Milli-Q water was used in most parts of the study, and it was generated by the Elga Purelab Flex 3 water purification system. In all cases ultrapure water was not available and, in these cases, tap water was used instead. HPLC grade methanol, acetonitrile, and formic acid were purchased from Thermo-fisher.

2.3 Methods

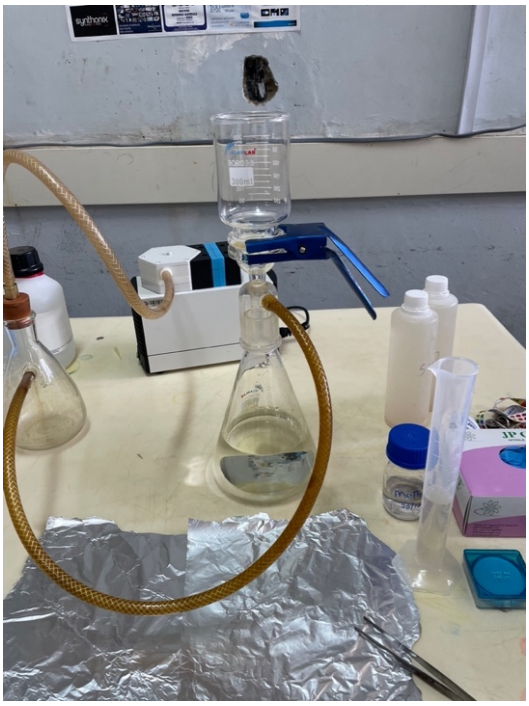
2.3.1 Measurement of physicochemical properties

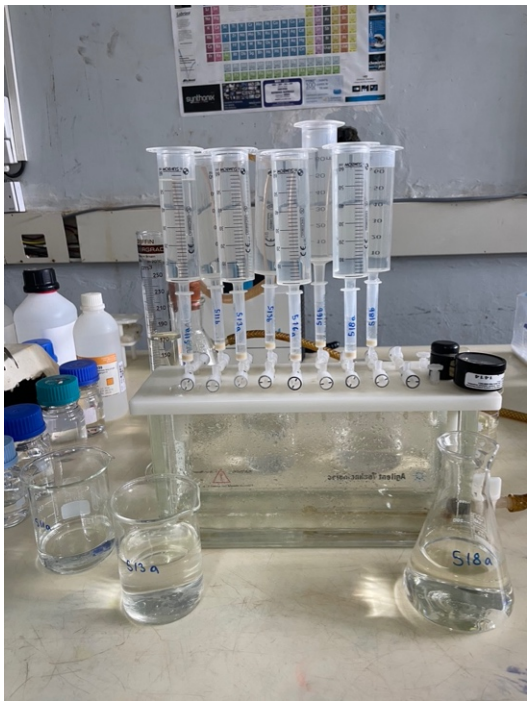
Physicochemical parameters of the water samples were measured by using the Isolab Waterproof portable pH, mV & Temperature pen tester. The measured parameters were pH, electrical conductivity (EC), and total dissolved solids (TDS). The measurements were done in the laboratory of JKUAT as soon as possible after the sample collection.

2.3.2 Sample preparation and extraction

The target compounds were extracted from the water samples using solid-phase extraction (SPE). For SPE extraction the water samples needed to be pre-filtered (Figure 8). Filtration is important to remove the particles from the sample that could otherwise clog the SPE cartridges. In filtration, the vacuum pump was used. Samples were filtered through two different 47 mm glass microfiber filter papers. First, through the 2.7 µm GF/D and then through the smaller 0.7 µm GF/F filter paper (Whatmat). In SPE extraction Oasis HLB 3cc SPE cartridges were used. Cartridges were first conditioned with at least 3 ml of 100 % methanol at a flow rate of 5 ml per minute and then washed with 3 ml Milli-Q water. After washing, the water samples were loaded (Figure 9). A 250 ml sample was loaded into the conditioned SPE cartridges and allowed to pass through at a flow rate of 5

ml/min. After loading, the samples were allowed to dry under the vacuum. Then samples were eluted using 3 ml of 5 methanol: acetonitrile (1:1) solution at a flow rate of 5 ml/min into clean KIMAX tubes. Eluted samples were evaporated to dryness using a Genevac miVAC DNA concentrator and reconstituted to 1 ml in a vial using Milli-Q water: methanol (90:10) dilution. Samples were then filtered into the HPLC vials through a 0.22 µm sterile cellulose syringe filter. After that, they were ready for the LC-MS/MS analysis run.





Figures 8 and 9. Pre-filtration of the water samples before the extraction with HBL SPE cartridges.

2.3.3 Method validation

For the LC-MS/MS run, the standard calibration for the determination of the concentrations of the target compounds is needed. Needed pharmaceutical standards were prepared as mentioned previously. Calibration curves were obtained for most of the analytes in the range of 5–400 µg/l with the exception of TET, OXT, and DOX, which were in the range of 50–400 µg/l due to their poor detection at lower concentrations. At least six points for each compound in the calibration curve remained, which is considered to be an acceptable amount of values (Moosavi et al. 2018). Appendix 1 contains the calibration curves for each compound. From the calibration curves, the coefficient of determination (r^2), the limit of detection (LOD), and the limit of quantification (LOQ) were obtained. The limit of detection (LOD) and the limit of qualification (LOQ) for each analyte were calculated from the calibration curves using 3 and 10 times the signal-to-noise ratio (S/N) respectively. LODs were calculated by using Equation 1 and LOQs by using Equation 2.

$$LOD = \frac{3*SD}{S} \quad (1)$$

$$LOQ = \frac{10*SD}{S} \quad (2)$$

Where *SD* is the standard deviation and *S* is the slope of the regression equation.

For the method validation, the recoveries (%) were also evaluated. For the evaluation blank water samples were spiked at concentrations of 50 µg/l, 100 µg/l, and 200 µg/l before and after extraction. Recoveries were calculated by comparing the concentrations of pre-extraction spiked samples to post-extraction spiked samples by using Equation 3.

$$Recovery = \frac{c(pre-extraction\ spike)}{c(post-extraction\ spike)} * 100\ \% \quad (3)$$

2.3.4 LC-MS/MS

The analyte identification and quantification were carried out in the LC-MS/MS system. LC/MS is often used in the analysis of pharmaceuticals because it enables the identification of polar organic pollutants such as pharmaceuticals down to relatively low concentrations levels from various matrices (Seifrtová et al. 2009). LC-MS/MS system consisted of an Agilent HP1100 LC system paired with a Micromass Quattro-Ultima mass spectrometer. The system was controlled by Mass Lynx software (Version 4.1). The used column in the liquid chromatography system was the Kinetex EVO C18 column (100 x 3.0 mm, 5 µm, 100A°). The mobile A phase consisted of 0.1 % formic acid in Milli-Q-water and the mobile phase B consisted of 0.1 % formic acid in acetonitrile (ACN). The gradient elution method was used, and it is presented in Table 4. The column temperature was 35 °C, the injection volume was 10 µl, and the flow rate was 0.450 ml/min.

TABLE 4. The gradient composition for the LC-MS/MS run used in the study. Solvent A was 0.1 % formic acid in Milli-Q water and solvent B was 0.1 % formic acid in acetonitrile.

Time	Solvent A %	Solvent B%	Flow (ml/min)	Pressure (bar)
0.00	90.00	10.00	0.45	400
2.00	90.00	10.00	0.45	400
5.00	60.00	40.00	0.45	400
8.00	0.00	100.00	0.45	400
10.00	0.00	100.00	0.45	400
10.01	90.00	10.00	0.45	400

The detector used was the Micromass Quattro-Ultima electrospray triple-quadrupole mass spectrometer (Manchester, UK). Nitrogen was used as both the desolvation gas (720 l/h) and as a cone gas. The collision gas was argon. The desolvation temperature and the source temperature were 120 °C and 350 °C, respectively. The optimized multiple reaction monitoring (MRM) parameters for the selected compounds analyzed are presented in Table 5.

TABLE 5. Optimized MRM parameters for the selected pharmaceutical compounds for LC-MS/MS analysis. RT = retention time.

Compound	RT (min)	Precursor ion $[M + H]^+$ (m/z)	Cone voltage (V)	Product ion 1 (m/z)	Collision energy (eV)	Product ion 2 (m/z)	Collision energy (eV)
AMX	1.11	366	20	349	10	114	20
AMP	2.11	350	20	192	15	106	15
TET	3.77	445	30	410	28	154	28
OXT	3.10	461	30	426	18	154	28
DOX	6.57	445	30	428	16	154	28
SMX	7.09	254	30	156	15	92	25
SDZ	2.73	251	30	92	25	156	15
SMZ	5.23	279	30	186	20	92	30
TMP	2.02	291	35	123	35	230	30
CBZ	8.09	237	30	194	20	192	20
3TC	0.88	230	17	112	17	95	45
NVP	7.05	267	30	226	30	197	45

2.3.5 Risk assessment for resistance selection

Risk assessment for the development of antimicrobial resistance was done by calculating the risk quotients (RQs) for each analyte if possible. Risk quotient calculation has often been used in the assessment of the ecological risk of pharmaceutical residuals (for example Verlicchi et al. 2012, Huang et al. 2018), but it is also suitable for the assessment of resistance selection. The RQs were calculated by using the measured concentrations of each analyte and their compound-specific predicted no-effect (PNEC) values for resistance selection (RS). The used $PNEC_{(RS)}$ values were those presented by Bengtsson-Palme and Larsson (2016). The RQs were calculated using Equation 4.

$$RQ = \frac{MEC}{PNEC(RS)} \quad (4)$$

Where the *RQ* is the risk quotient, *MEC* is the measured environmental concentration in the sample and $PNEC_{(RS)}$ is the predicted no-effect concentration

for resistance selection. $RQ \geq 1$ means a high risk, $1 > RQ \geq 0.1$ medium risk, and $RQ < 0.1$ low risk for resistance selection. (Hernando et al. 2006)

3 RESULTS

3.1 Sample water physicochemical parameters

The measured physicochemical parameters of the sampling waters are presented in Table 6. Physicochemical properties were measured during the first sampling round. From sampling points 3 and 4 physicochemical properties were not measured due to them being part only the second sampling round. The pH of the samples ranged from 4.8 to 5.8, total suspended solids (TDS) from 38 ppm to 712 ppm, and electrical conductivity (EC) from 58 µs to 1058 µs. The highest TDS and EC were measured from sampling point 9. In samples from Thiririka River, TDS, and EC values were generally lower than in the samples from open drains. Also, the pH of river water was lower than in most of the open drains.

TABLE 6. Measured physicochemical properties of the surface water samples from the sampling points in the study. TDS = total dissolved solids, EC = electrical conductivity, n.m = not measured.

Sample	pH	TDS (ppm)	EC (µs)
1. Thririka River	4.8	38.0	57.5
2. Thririka River	4.8	77.5	116.5
3. JKUAT drain	n.m	n.m	n.m
4. JKUAT pond	n.m	n.m	n.m
5. Open drain	5.3	208.5	312.0
6. Open drain	5.6	428.0	639.0
7. Open drain	4.7	97.0	147.0
8. Open drain	5.3	112.5	169.5
9. Open drain	5.7	712.0	1057.5
10. Open drain	5.8	521.0	778.5

3.2 Method Validation Parameters

The LC-MS/MS method validation parameters for quality assurance and control are presented in Table 7. The linearity of the calibration curves (r^2) exceeded 0.99 for all the target compounds. Calibration curves for each compound are given in Appendix 1. The target compound mean recovery values ranged from 12 to 95 %, with OXT having the lowest recovery and 3TC having the highest recovery. The LOD and LOQ values were in the ng/l range and varied between compounds.

TABLE 7. LC-MS/MS method validation results. RSD = relative standard deviation, LOD = limit of detection, LOQ = limit of quantification.

Compound	r^2	Recovery (%) (mean ± RSD)	LOD (ng/l)	LOQ (ng/l)
AMX	0.997	32 ± 0.3	0.94	3.15
AMP	0.998	48 ± 0.4	0.85	2.84
TET	0.995	39 ± 0.4	1.74	5.84
OXT	0.996	12 ± 1.2	1.72	5.73
DOX	0.992	66 ± 0.2	2.55	8.50
SMX	0.994	87 ± 0.1	1.43	4.78
SDZ	0.991	91 ± 15	1.76	5.86
SMZ	0.998	84 ± 0.1	0.73	2.44
TMP	1.000	62 ± 0.5	0.39	1.31
CBZ	0.998	70 ± 0.5	0.86	2.86
3TC	0.999	95 ± 0.1	0.62	2.08
NVP	0.999	78 ± 0.3	0.41	1.37

3.3 Occurrence and concentrations of detected antimicrobials

The presence of ten antibiotics (AMX, AMP, TET, OXT, DOX, SMX, SDZ, SMZ, TMP, CBZ) and two antiretroviral drugs (3TC, NVP) in surface water in Juja, Kenya was investigated. The majority of samples were collected from unlined open drains, with a few samples taken from the Thiririka River and a pond inside JKUAT University. Concentrations of the compounds varied significantly among sampling points and compounds. There were also differences in the concentrations between the first and the second rounds of sampling, with results presented separately for each round. The combined results from the open drains studied in both sampling rounds are presented at the end.

In the first sampling 9 out of 12, target compounds were detected in the samples. OXT, SDZ, and SMZ were not found in any of the sampling points, whereas SMX, TMP, 3TC, and NVP were detected in all the sampling points. A summary of detection frequencies (%) with mean, median, minimum, and maximum values of the measured concentrations of the target compounds from the first sampling round are present in Table 8, whereas the concentration of each compound in different sampling points is given in Table 9. The compounds were detected in ng/l level and the concentrations ranged from 17 ng/l to 3166 ng/l. The mean concentration of selected compounds was 276 ng/l, and the total sum of concentration was 13 505 ng/l.

TABLE 8. The detection frequencies (DF %) and mean ± standard deviation (SD), median, minimum (min), and maximum (max) concentrations (ng/l) of the target compounds in the first sampling round. n.d = not detected.

Compound	DF %	Mean ± SD	Median	Min	Max
AMX	50	59.0 ± 2.5	58.8	55.8	62.6
AMP	12.5	32.4	32.4	32.2	32.4
TET	12.5	396.9	396.9	396.9	396.9
OXT	0	n.d	n.d	n.d	n.d
DOX	75	404.6 ± 32.6	393.0	371.3	459.2
SMX	100	316.8 ± 153.2	295.3	131.3	555.6
SDZ	0	n.d	n.d	n.d	n.d
SMZ	0	n.d	n.d	n.d	n.d
TMP	100	125.9 ± 148.2	69.6	35.7	507.0
CBZ	50	61.1 ± 15.5	70.8	17.2	73.3
3TC	100	695.2 ± 948.4	378.7	102.9	3166.4
NVP	100	138.5 ± 102.3	62.9	58.2	306.2

Lamivudine was detected with a 100 % detection frequency, with the highest maximum concentration of 3166 ng/l and a mean concentration of 695 ng/l. 3TC is one of the first-line antiretroviral drugs used for the treatment and prevention of HIV infections in Kenya (NASCOP 2022). 3TC is also highly soluble in water (PubChem 2024), resulting that it is often detected with high concentrations in water samples. Nevirapine was also detected at a detection frequency of 100 % with a mean and maximum concentration of 58 ng/l and 306 ng/l, respectively. Like 3TC, NVP is also one of the first-line ART drugs used in Kenya (NASCOP 2022). NVP is less soluble in water and absorb more easily to soil and sediments. It is also excreted mostly as metabolites of the parent compound, with only 2.7 % being excreted unchanged (Riska et al. 1999), which could also explain why lower concentrations of it are detected.

Sulfamethoxazole and trimethoprim were also detected at a 100 % detection frequency with concentrations ranging from 131 ng/l to 556 ng/l and 36 ng/l to 507 ng/l, respectively. SMX is one of the most consumed antibiotics in Kenya and it is used with TMP in combination drug (cotrimoxazole) to treat and prevent coinfections related to HIV (Ngumba et al 2016a). The ratio of SMX to TMP in cotrimoxazole is 5:1 (NASCOP 2022), which can explain why higher concentrations of SMX were detected compared to TMP. SMX has also a relatively high water solubility and low sorption capacity (Boxall et al. 2002, Ozumchelouei et al. 2020), resulting in it being detected in water samples with relatively high concentrations. TMP has a higher K_d value, and it absorbs in the soil more easily (Ngumba et al. 2020), leading to lower concentrations detected in the water phase.

Doxycycline was detected with a 75 % detection frequency, with concentrations ranging from 371 ng/l to 459 ng/l. DOX is one of the most popular antibiotics used in Kenya for a variety of purposes. Its use in agriculture and veterinary purposes is also high. (Kariuki et al. 2011) High consumption of DOX can lead to relatively high concentrations of it being detected in the environment. Amoxicillin and carbamazepine were detected in half of the sampling points with the concentration ranging from 56 ng/l to 63 ng/l and 17 ng/l to 73 ng/l, respectively. Ampicillin was only detected in one sampling point, at a concentration of 32 ng/l. CBZ has relatively low solubility in water (PubChem), which can be a reason for its low concentrations in the samples. AXM and AMP are beta-lactams and due to the beta-lactam ring in their structure they have poor stability, and they are easily hydrolyzed resulting in their generally low detection in the environment (Cha et al. 2006, Felis et al. 2020, Addis et al. 2024). pH of the water and for example presence of metal ions also influence a lot to the fate of AMX and AMP in the environment (Addis et al. 2024).

Tetracycline was detected only in one sampling point, with a concentration of 398 ng/l. Tetracyclines (TCs) have usually good sorption capacity resulting in their presence in the aqueous phase being relatively low and they are more likely to be found in the soil and sediments (Christian et al. 2003, Seifrtová et al. 2009, Scaria et al. 2021). Various factors, such as cations in the environment, can also affect the absorption of tetracyclines into the soil (Felis et al. 2020, Addis et al. 2024). This can explain the lower concentration of TET being detected in the aqueous samples. Oxytetracycline, sulfadiazine, and sulfamethazine were not detected during the first sampling round. OXT belongs to the group of tetracyclines, and similarly to TET, it is relatively persistent in the soil (Li et al. 2008, Scaria et al. 2021). SDZ and SMZ have stronger sorption behavior compared to SMX, which can result in them not being detected from the aqueous phase as much (Bailey et al. 2016).

TABLE 9. Concentrations (ng/l) of selected compounds in different sampling points in the first sampling round. LOD = limit of detection, n.d = not detected.

Compound	1. Thririka River	2. Thririka River	5. Open drain	6. Open drain	7. Open drain	8. Open drain	9. Open drain	10. Open drain
AMX	n.d	55.7±0.5	57.9±1.0	62.6±0.5	n.d	59.7±3.4	n.d	n.d
AMP	n.d	n.d	n.d	n.d	n.d	n.d	n.d	32.4
TET	396.9	n.d	n.d	n.d	n.d	n.d	n.d	n.d
OXT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
DOX	371.3±4.6	374.2± 0.9	n.d	395.1± 0.1	n.d	390.9± 0.2	459.2± 0.04	437.2± 6.4
SMX	131.3±7.5	175.2± 10.7	485.4± 54.9	555.6± 40.1	367.56± 65.4	164.4± 26.7	432.0± 12.2	223.0± 2.9
SDZ	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

SMZ	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TMP	35.7	43.2±6.3	507.0± 3.6	143.6± 43.9	98.9± 64.8	87.3± 2.7	51.8± 18.4	39.5± 3.9
CBZ	<LOD	<LOD	70.8± 28.2	73.3± 12.1	<LOD	39.2± 1.6	<LOD	17.2
3TC	102.9± 2.3	148.8± 1.9	333.2± 4.2	424.1± 0.3	293.9± 5.3	424.8± 41.3	3166.4± 7.5	667.3± 25.5
NVP	60.7± 0.5	61.8± 3.3	306.2± 2.8	275.9± 9.1	220.5	60.7± 0.01	58.2	64.1± 4.2
Total	1098.7	860.0	1760.6	1930.2	980.9	1227.2	4167.5	1480.7

The concentrations of target compounds in different sampling places during the first sampling round are illustrated in Figure 10. The highest total concentration of target compounds, 4168 ng/l, was measured in open drain 9. Open drain 9 was located in a densely populated area, and based on the field observation, it is likely that nearby houses were discharging their wastewater into the open drain, leading to higher levels of pharmaceuticals in the environment. The concentration of 3TC was particularly high in open drain 9 (3166 ng/l). Samples from Thiririka River and open drain 7 had the lowest total concentrations of the compounds. The total concentrations in river samples were 1099 ng/l and 860 ng/l whereas the total concentration in open drain 7 was 981 ng/l. The concentrations of selected compounds were generally lower in river samples compared to samples from open drains, which can be due to higher dilutions of compounds in the river water. For example, the mean concentration of SMX in open drains was almost 150 % higher compared to river. However, TET was detected only from one of the river samples and not from any of the open drains.

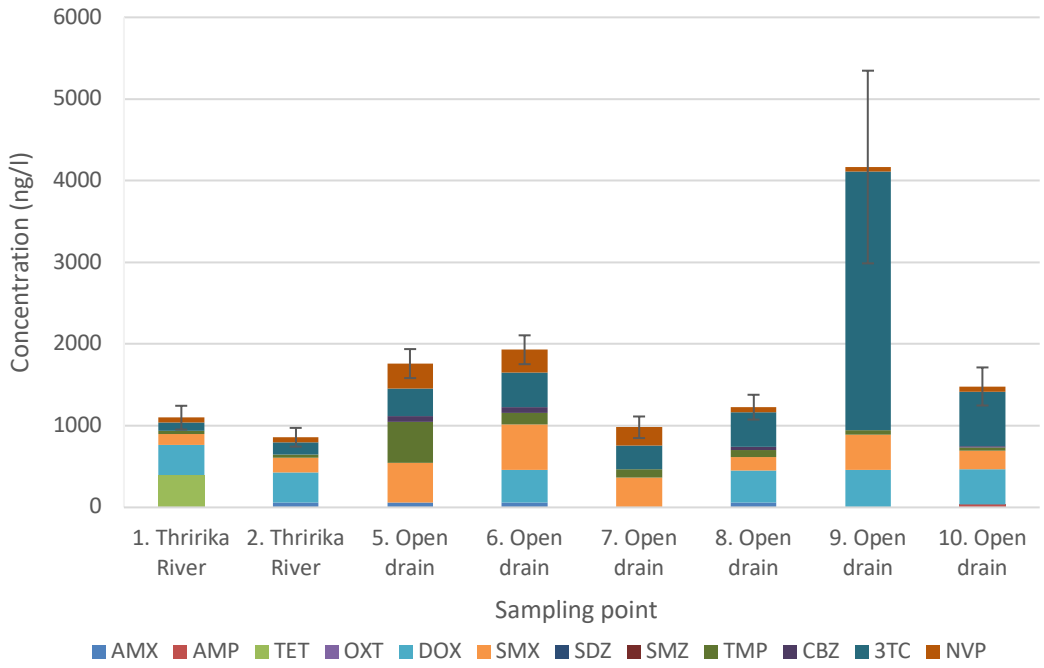


Figure 10. The concentrations (ng/l) of target compounds in different sampling points in the first sampling.

In the second sampling, 9 out of 12 target compounds were detected in the samples. AMP, OXT, and SDZ were not detected in any of the sampling points whereas DOX, SMX, TMP, 3TC, and NVP were detected in all the sampling points. A summary of detection frequencies with the mean, median, minimum, and maximum values of concentration of selected compounds from the second sampling round are present in Table 10, while the concentrations of each compound at different sampling points from the second sampling are given in Table 11. The concentrations of the compounds ranged from 46 ng/l to 4269 ng/l. The mean concentration of selected compounds was 477 ng/l, and the total sum of concentration was 30 977 ng/l, both values being considerably higher compared to the first sampling rounds.

TABLE 10. The detection frequencies (DF %) and mean ± standard deviation (SD), median, minimum (min), and maximum (max) concentrations (ng/L) of the target compounds in the second sampling round. LOD = limit of detection, n.d = not detected.

Compound	DF %	Mean ± SD	Median	Min	Max
AMX	87.5	56.6 ± 1.2	56.5	54.4	60.6
AMP	0	n.d	n.d	n.d	n.d
TET	37.5	425.5 ± 23.1	416.6	405.1	454.8
OXT	0	n.d	n.d	n.d	n.d
DOX	100	496.2 ± 27.7	492.8	451.1	530.45
SMX	100	2012.6 ± 1365.0	2117.6	276.8	4268.9

SDZ	0	<LOD	<LOD	<LOD	<LOD
SMZ	87.5	94.3 ± 27.7	81.8	67.6	149.4
TMP	100	116.6 ± 62.4	98.3	46.1	241.4
CBZ	87.5	193.4 ± 164.2	86.9	47.6	442.1
3TC	100	558.9 ± 669.1	235.0	116.7	2179.2
NVP	100	221.5 ± 42.5	222.3	155.7	304.2

Sulfamethoxazole was found to have the highest maximum concentration of 4269 ng/l with a detection frequency of 100 %. SMX also had the highest measured mean value of 2013 ng/l of the compounds. The mean concentration of SMX was over five times higher compared to the first sampling round. Trimethoprim was also detected at all the sampling points, with maximum and mean concentrations of 241 ng/l and 117 ng/l, respectively. In the first sampling, TMP was detected with a slightly higher concentration. Doxycycline was also detected with a detection frequency of 100 %. The measured concentration of DOX ranged from 451 ng/l to 530 ng/l. DOX was also commonly detected in the first sampling but at lower concentrations.

Both selected antiretrovirals were also detected with a detection frequency of 100 %. Lamivudine was measured at concentrations ranging from 117 ng/l to 2179 ng/l whereas nevirapine was measured at concentrations from 156 ng/l to 304 ng/l. 3TC and NVP were also some of the most common compounds in the first sampling. In the first sampling, the concentration of 3TC was slightly higher and the concentration of NVP was slightly lower. Amoxicillin, sulfamethazine, and carbamazepine were all detected with a detection frequency of 87.5 % with concentrations ranging from 54 ng/l to 61 ng/L, 68 ng/l to 149 ng/l, and 48 ng/l to 442 ng/l, respectively. Detection frequency and measured concentrations of AMX were similar to those of the first sampling. In the first sampling, SMZ was not detected and CBZ was detected with lower concentrations. Tetracycline was detected with a detection frequency of 37.5 % at concentrations ranging from 405 ng/l to 455 ng/l. Concentrations of TET were similar in the first sampling. AMP, OXT, and SDZ were not detected in any of the samples. In the first sampling, OXT and SDZ were also not detected, and AMP was only detected in one of the sampling points with a low concentration.

TABLE 11. Measured concentrations (ng/l) of each target compound in different sampling points in the second sampling round. LOD = limit of detection, n.d = not detected.

Compound	4. JKUAT pond	3. JKUAT drain	5. Open drain	6. Open drain	7. Open drain	8. Open drain	9. Open drain	10. Open drain
AMX	54.9±2.2	56.9±2.7	57.0±2.0	54.4±0.4	n.d	56.6	56.2±1.2	60.6±2.5
AMP	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TET	n.d	n.d	454.8	n.d	416.6	405.1	n.d	n.d

OXT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
DOX	491.8± 4.2	493.7± 5.1	530.5± 3.6	474.4± 10.1	511.1± 16.8	490.0± 18.1	526.6± 27.9	496.2± 24.7
SMX	276.8± 105.0	1414.6± 3.0	2906.9± 264.5	371.9± 41.7	3093.5± 107.1	4268.9± 194.3	948.2± 37.0	2820.6± 80.8
SDZ	n.d	n.d	<LOD	n.d	n.d	<LOD	<LOD	n.d
SMZ	114.9± 2.7	67.6±1.8	149.4± 25.8	75.8±4.4	69.0	81.8±7.1	n.d	101.6± 3.6
TMP	78.4±5.9	100.4± 36.3	96.3±2.4	66.6±1.5	193.8± 5.2	241.4± 6.1	46.1±9.9	109.5± 22.9
CBZ	427.6± 2.4	55.0±2.2	236.4± 87.2	86.9±9.5	<LOD	57.9±3.3	47.6± 17.1	442.1± 35.7
3TC	116.7± 3.2	193.9± 6.2	249.6± 4.3	220.4± 6.6	160.3± 8.7	336.1± 2.8	2179.3± 145.5	1014.8± 28.6
NVP	155.7± 9.5	212.4± 2.7	215.8± 23.2	234.7± 33.5	173.4± 7.9	246.7± 32.7	228.9± 24.7	304.6± 11.9
Total	1716.7	2594.4	4897.0	1585.1	4617.3	6184.4	4032.9	534.6

The concentrations of target compounds in different sampling points during the second sampling round are illustrated in Figure 11. The highest total concentration, 6184 ng/l, was measured from open drain 8, while the lowest concentration, 1585 ng/l, was found from open drain 6. Open drain 8 was located near the old WWTP in the area. Measured concentrations from the JKUAT pond were also relatively low compared to samples from open drains, possibly due to higher dilution of compounds in the water. In most sampling points, SMX was the most abundant compound. Similarly to the first sampling, 3TC was detected with a considerably high concentration (2179 ng/l) in open drain 9.

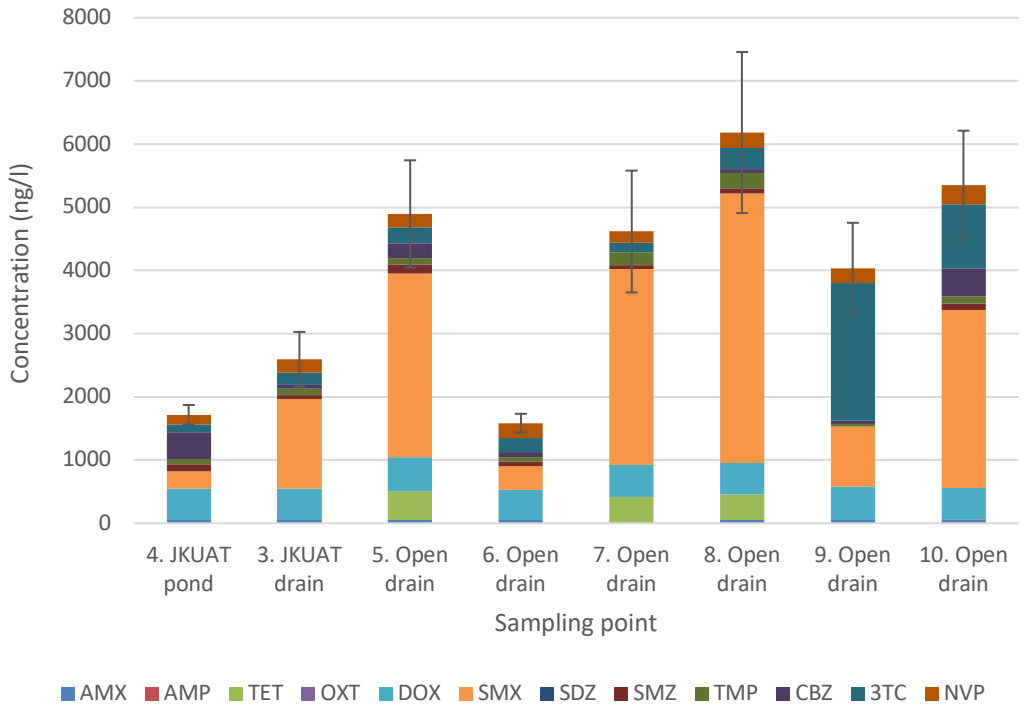


Figure 11. The measured concentrations (ng/l) of target compounds in different sampling points in the second sampling.

Open drains in Juja (sampling points 5 – 10) were sampled during both rounds of sampling. A summary of the combined results from the open drain samples is presented in Table 12. Overall, the detection frequency in open drains ranged from not detected to 100 %, while concentration ranged from 17 ng/l to 4269 ng/l. SMX had the highest mean (1387 ng/l) and maximum concentration (4269 ng/l) with a detection frequency of 100 %. TMP, 3TC, and NVP had also 100 % detection frequency with mean concentrations of 140 ng/l, 789 ng/l, and 199 ng/l, respectively. DOX was detected with a detection frequency of 83 % and a mean concentration of 471 ng/l. AMX, TET, SMZ, and CBZ had detection frequencies between 25 – 69 % with mean concentrations being 58 ng/l, 426 ng/l, 96 ng/l, and 119 ng/l, respectively. AMP had the lowest detection frequency of 8 % with a concentration of 32 ng/l while SDZ and OXT were not detected in any of the open drains.

TABLE 12. The mean ± standard deviation (SD), median, minimum (min) and maximum values of measured concentrations (ng/l) of target compounds with their detection frequencies combined from open drain samples from both sampling rounds. LOD = limit of detection, n.d = not detected.

Compound	DF %	Mean ± SD	Median	Min	Max
AMX	67	58.1 ± 2.5	57.5	54.4	62.6
AMP	8	32.4	32.4	32.4	32.4
TET	25	425.5 ± 21.1	416.6	405.1	454.8

OXT	0	n.d	n.d	n.d	n.d
DOX	83	471.1 ± 47.6	482.2	390.9	530.5
SMX	100	1386.5 ± 1387.7	520.5	164.4	4268.9
SDZ	0	<LOD	<LOD	<LOD	<LOD
SMZ	42	95.5 ± 29.1	81.8	69.0	149.4
TMP	100	140.1 ± 124.0	97.6	39.5	507.0
CBZ	75	119.1 ± 128.7	70.8	17.2	442.1
3TC	100	789.2 ± 893.7	380.1	160.3	3166.4
NVP	100	199.1 ± 87.3	224.7	58.2	306.2

The concentrations of target compounds in different open drain samples from both sampling rounds are presented in Figure 12. The highest total concentration was measured from open drain 9, which was located in a densely populated area in Gachororo. The concentrations from open drains 5 and 8 were almost as high. Open drain 5 was located next to the road entering JKUAT, while open drain 8 was located near the old WWTP. The lowest measured concentration was from open drain 6, located in Muchatha. Various factors affect the occurrence of antibiotics and pharmaceuticals in the environment. In addition to the properties of the compound, also the different excretion rates of the compounds in their unchanged form and as metabolites affect. Environmental conditions, such as pH, temperature, and other compounds in the water, also influence the fate of pharmaceuticals in the surface water. (Kümmerer 2009, Harrower et al. 2021) Various processes, such as biodegradation or photodegradation, can also impact the fate of pharmaceuticals and reduce their concentration in the environment. (Ebele et al. 2017) This is why the prediction of the occurrence and fate of pharmaceuticals in the environment can be challenging. In discussion, the results of this study will be further discussed and compared to previous studies.

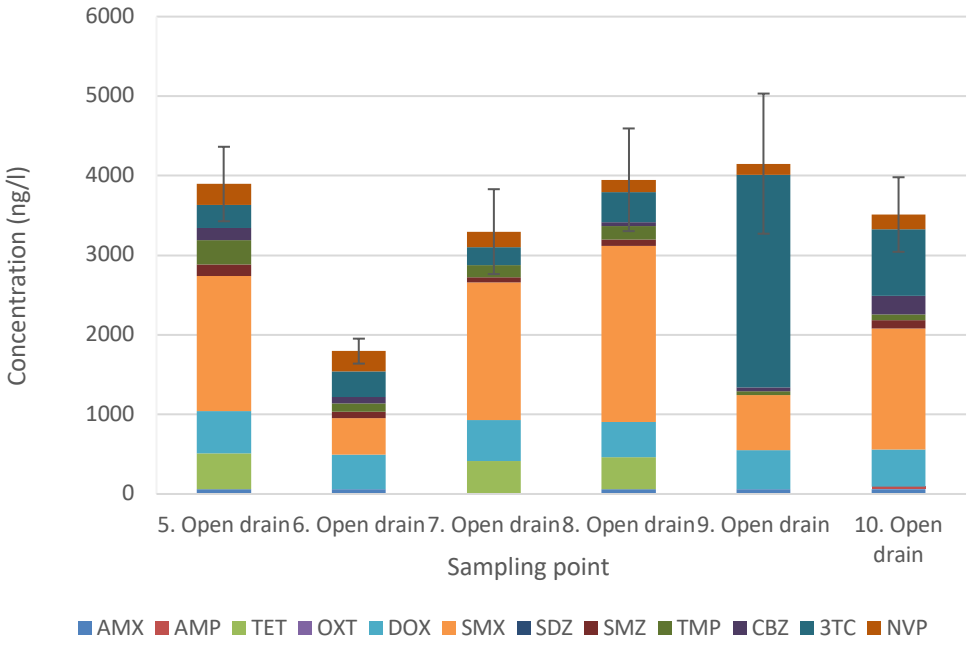


Figure 12. Total measured concentrations (ng/l) of the target compound in open drain samples combined from the first and second sampling rounds.

3.4 Antibiotic resistance risk assessment

The risks for antibiotic resistance selection for each target compound of the study are presented in Table 13. The risks were calculated based on the lowest and highest measured concentrations of the compounds found in the samples. Values were compared to PNEC values for resistance selection, which were obtained from the study by Bengtsson-Palme & Larsson (2016). TMP had a low to high risk and SMX a low to medium risk for resistance. AMC, AMP, TET, and DOX had medium risks for resistance selection. For OXT, SDZ, SMZ, CBZ, 3TC, or NVP the risks could not be calculated due to missing $PNEC_{(RS)}$ values or compounds not being detected in the samples. It is important to notice that the RQ considers only the parent molecule of the compounds. Only a portion of the compounds are excreted unchanged, so it would also be useful to measure the metabolites and transformation products of the compounds.

TABLE 13. The risk for antibiotic resistance selection for each target compound. MEC = measured environmental concentrations, $PNEC_{(RS)}$ = predicted no-effect concentration for resistance selection, n.d = not detected, n.r = not reported, RQ = risk quotient. RQ ≥ 1 = high risk, 1 > RQ ≥ 0.1 = medium risk, and RQ < 0.1 = low risk for resistance selection. (Hernando et al. 2006)

Compound	MEC (µg/l)	$PNEC_{(RS)}$ (µg/l)*	RQ	Risk
AMX	0.054 – 0.063	0.25	0.22 – 0.25	medium
AMP	0.032	0.25	0.13	medium
TET	0.41 – 0.45	1	0.41 – 0.45	medium

OXT	n.d	0.5	n.d	
DOX	0.37 – 0.53	2	0.19	medium
SMX	0.13 – 4.27	16	0.008 – 0.27	low to medium
SDZ	<LOD	n.r	n.d	
SMZ	0.068 – 0.15	n.r	n.r	
TMP	0.036 – 0.51	0.5	0.074 – 1.01	low to high
CBZ	0.017 – 0.44	n.r	n.r	
3TC	0.10 – 3.17	n.r	n.r	
NVP	0.058 – 0.31	n.r	n.r	

* Bengtsson-Palme & Larsson (2016)

4 DISCUSSION

Most of the antibiotics and antiretrovirals that were studied were detected in the samples. The concentrations and detection frequencies varied among different compounds. As mentioned, the environmental occurrence and fate of pharmaceuticals are highly influenced by their physicochemical properties. In this study, compounds with higher solubility to water, such as SMX, were generally found with higher concentrations, whereas the compounds, with higher sorption capacity, like tetracyclines, were measured with lower concentrations. The activities of humans and the consumption amount of pharmaceuticals also have an impact on environmental concentrations. Commonly used antibiotics and antiretrovirals in Kenya were detected with the highest concentrations in the study. These included especially SMX, TMP, and DOX as well as both antiretrovirals studied.

There were also differences observed between sampling points, which can be due to varying loads and sources of contamination between different locations Environmental conditions of sampling points may also influence the occurrence of compounds. In the study, the sampling points with the highest measured concentrations of the compounds were likely from areas with higher contamination. For example, the concentrations near the old WWTP and in the places where nearby households probably discharged their wastewater were relatively high. Detected concentrations were also higher in the areas that were more densely populated. A significantly high occurrence of 3TC was found in open drain 9 during both sampling rounds, which may be attributed to higher use of it in that area. The total concentrations in river samples were probably lower due to the higher dilution of compounds in the river water compared to open drains. Additionally, there may be lower amounts of pharmaceuticals entering the river compared to the open drains analyzed. During the first sampling, TET was detected with a higher concentration from the river than from the open drains. The river sampling point was surrounded by fields, and based on the observation, there might have been agricultural activities in the area. TET is highly used for agricultural purposes, which could be a reason for higher concentrations of it detected at that point.

There were also some differences between the first and second sampling. The most detected compounds were similar in both sampling rounds, but there was variation in the concentrations. The total sum of concentrations was almost two times higher in the second sampling compared to the first sampling. Especially, the concentration of SMX was higher during the second sampling. Differences in concentrations may have been caused by seasonal variation and changes in rainfall amounts between the sampling rounds. The rainfall amount for those months could not be found, so this is based solely on field observations. Additionally, the environmental conditions may have been slightly different. Water parameters could only be measured during the first round of sampling, therefore any possible differences caused by them cannot be evaluated. The sampling rounds were carried out relatively close to each other, resulting in consumption amounts being probably roughly the same during both samplings. Therefore, the consumption amounts probably do not explain the differences in the results, although they may have some influence.

The result also showed that the risk for resistance selection varied from low to high risk depending on the compound. The concentration of the compound was not directly related to the risk of resistance. For example, TMP was estimated to have the highest risk for resistance development, despite having significantly lower concentrations compared to for example SMX. Next, the results of this study are compared with previous research in the region, as well as to other studies from Kenya and other locations.

4.1 Comparison of the results with the previous study from Juja

The results of this study are compared with the values reported in a previous study from the same region. Muriuki et al. (2020) conducted a study on the occurrence and risk of selected antibiotics and antiretrovirals in Juja town in August 2019. The study and methods used were generally similar to this study, although there were some differences in the sample types and sampling points selected. Similar unlined open drains from different areas of Juja town were sampled as in this study. In addition, in Muriuki's study, some of the open drains were also concrete-lined drains. Muriuki et al. (2020) also collected samples from the JKUAT wastewater treatment plant and the Ndarugu River, where effluents from the treatment plant were discharged. In this study, samples were not collected from the wastewater treatment plant, as it was no longer operational. However, there was one sampling point near the old WWTP. The wastewater treatment plant in JKUAT served about 20 000 people and used stabilization ponds for treating the collected wastewater (Muriuki et al. 2020). In the Muriuki et al. (2020) study, there were also sediment samples collected. The selected compounds in Muriuki's study were ciprofloxacin (CIP), trimethoprim (TMP), norfloxacin (NOR), sulfamethoxazole (SMX), amoxicillin (AMX), lamivudine (3TC), zidovudine (ZDV), and nevirapine (NVP). The measured concentration in the study by Muriuki et al. (2020), for the same compounds studied in this study (AMX, SMX, TMP, 3TC, NVP), are compared with the result in this study. The mean, minimum, and maximum concentrations measured from

open drains in Muriuki's study for those compounds are presented in Table 14. For comparison, the same results from this study are included in Table.

TABLE 14. Reported concentration (ng/l) of selected antibiotic and antiretroviral drugs from open drain samples in Muriuki et al. (2020) and this study. SD = standard deviation.

	Muriuki et al. (2020)			This study		
Compound	Mean ± SD	Min	Max	Mean ± SD	Min	Max
AMX	1200±2000	300	3300	58±3	54	63
SMX	108000±200000	5700	506000	1387±1388	164	4269
TMP	37700±60000	4600	479000	140±124	40	507
3TC	532000±300000	124000	913000	789±894	160	3166
NVP	3260±2000	1400	145000	199±87	58	306

Compared to this study, the reported concentrations in open drain samples in the Muriuki et al. (2020) study were considerably higher for all the compounds. The concentrations of compounds ranged from 300 ng/l to 913 000 ng/l, while in this study, the range was from 32 ng/l to 4269 ng/l. Similarly to this study, in the open drain samples, the SMX and TMP were the most detected in Muriuki's study. The reported mean concentration of SMX was in Muriuki et al. (2020) study 108 000 ng/l, whereas in this study it was 1387 ng/l. TMP was detected with a mean concentration of 37 700 ng/l in Muriuki's study and 140 ng/l in this study. As said, the high detection of SMX and TMP in surface water samples in both studies can be attributed to their high consumption and use in the treatment of HIV/AIDS-related infections. AMX was detected with the lowest concentrations in both studies, with a mean concentration of 1200 ng/l in Muriuki's study and 58 ng/l in this study. The low concentration of AMX could be due to lower consumption and hydrolysis of the beta-lactam ring. Both antiretrovirals were detected with high concentrations. In Muriuki's study, the mean concentration of 3TC was 532 000 ng/l and the mean concentration of NVP was 3260 ng/l. The mean concentrations of 3TC and NVP in this study were 789 ng/l and 199 ng/l, respectively. In both studies, the concentration of 3TC was higher than the measured concentrations of NVP. This can be affected by the different properties, consumption amounts, and excretion rates of those compounds. Only 2.7 % of NVP is excreted as a parent compound, while 70 % of 3TC is excreted unchanged (Ngumba et al. 2016a). The order of the concentrations of the compounds was similar in both studies, but concentrations varied significantly.

In both studies, there were differences in concentrations between different sampling points. Open drains were not the exact same in this study and in the Muriuki et al. (2020) study. However, similar to this study, the highest concentrations from open drains were detected in areas that were densely populated and more slum-like. In the Muriuki et al. (2020) study, there were also differences between different types of open drains. In unlined open drains, there was more accumulation of compounds in the sediment compared to lined open drains, which can affect the results. Reported concentrations in the river were lower than concentrations from the open drains in both studies. In the Muriuki

et al. (2020) study, the highest concentration of 3TC detected in the river was 70 300 ng/l, whereas in this study the maximum concentration of 3TC in the river was 149 ng/l. The maximum concentrations of AMX and TMP were 700 ng/l and 7200 ng/l in Muriuki's study, whereas SMX and NVP were not detected in any of the river samples. In this study, the maximum concentrations of AMX, SMX, TMP, and NVP in the river were 56 ng/l, 175 ng/l, 43 ng/l, and 62 ng/l, respectively. In Muriuki's study, samples were from the Ndarugu River, and in this study from the Thiririka River, which can affect the results and explain some of the differences. Ndarugu River was the discharge point for effluents from the old WWTP. In Muriuki's study, the WWTP was named as the primary point source of pollution. This can explain the higher concentration detected in the Ndarugu River compared to the Thiririka River. Pollution of the Thiririka River mainly comes from non-point sources. In Muriuki's study, the role of non-point wastewater discharge was also recognized to have a significant impact.

Muriuki et al. (2020) calculated RQ values for both ecological and antibiotic resistance development risks. In this study, the ecological risk values were not calculated, so only the risk values for resistance selection are going to be compared. In Muriuki's study, the risk for resistance development was determined based on maximum environmental concentrations and $PNEC_{(RS)}$ values by Bengtsson-Palme & Larsson (2016), similar to this study. In Muriuki's study, the RQ values ranged from n.r to 957 in drain water. The RQ values for 3TC and NVP could not be calculated either in this or in the Muriuki et al. (2020) study. Similarly to this study, TMP had the highest RQ value and risk for resistance selection. However, in this study, the RQ value of TMP was considerably lower (RQ = 1.01) compared to Muriuki's study (RQ = 957) In Muriuki's study, the RQ value for SMX was 32, whereas in this study, it was only 0.27. AMX has an RQ value of 13 in Muriuki's study, while in this study, the RQ value was 0.25. Overall, the RQ values were considerably higher in Muriuki's study compared values in this study.

Many things that can explain the differences between the studies. One factor is the time of the sampling. Muriuki et al. (2020) study was conducted in the dry month of August, when the river flow was low, and a significant proportion of water came from WWTP. During the sampling, the open drains in the study area were mainly composed of untreated wastewater from households. This results in lower dilution of compounds compared to this study when the amounts of rainfall were likely higher. Additionally, the selection of sampling points and different sampling locations can impact the results. Some areas may be more contaminated or receive more wastewater from households. Furthermore, the properties and conditions of the environment can also have an effect. For example, the pH values reported in the Muriuki et al. (2020) study were higher than the pH values in this study. pH, as well as other environmental parameters, can influence the presence and fate of compounds (Lertpaitoonpan et al. 2009). Additionally, the consumption amounts of the compounds may have varied between studies, but this is difficult to determine accurately due to insufficient data on consumption amounts.

4.2 Comparison of the results with other studies

Measured concentrations in this study are also compared to reported values from the other locations. The concentrations are compared to reported values from surface waters in Kenya and Africa as well as some European and Asian countries.

Among sulfonamides, SMX is often detected with the highest concentrations in aqueous samples (aus der Beek et al. 2016, Fedaku et al. 2019, Chemtai et al. 2023, Hawash et al. 2023). It is also one of the most studied antibiotics. In this study concentration of SMX varied between 131 ng/l to 4269 ng/l. Other studies from Kenya have reported maximum concentration of SMX in surface water as 38 850 ng/l (K'oreje et al. 2016), 56 000 ng/l (Kairigo et al. 2020a), 97 000 ng/l (Kairigo et al. 2020b), 13 000 ng/l (Ngumba et al. 2016a), 274 ng/l (Chemtai et al. 2023), and 6840 ng/l (Ngigi et al. 2020). Most of the reported concentrations are significantly higher than the concentration in this study, except for the reported values in the studies by Chemtai et al. (2023) and Ngigi et al. (2020). The samples in these studies are mostly from river water, with variations in sampling locations and times. The measured concentrations of SMX were also lower compared to reported values from different parts of Africa, but higher than those reported from Europe and Asia. Maximum concentrations for SMX in surface water in Africa have been reported as 11 800 ng/l in Zambia (Ngumba et al. 2020), 10 568 ng/l in South Africa (Segura et al. 2015), 9640 ng/l in Ghana (Sequra et al. 2015), and 53 828 ng/l in Mozambique (Segura et al. 2015). In comparison, maximum concentrations measured in Europe and Asia were significantly lower, 68 ng/l (aus der Beek et al. 2016) and 258 ng/l (aus der Beek et al. 2016), respectively. In a study by Ngumba et al. (2016b) from Finland, SMX was detected with 25 ng/l concentration from surface water.

In this study, SMZ was detected in less than half of the samples, with concentrations ranging from 68 ng/l to 149 ng/l, whereas SDZ was not detected in any of the samples. In many other studies, the concentrations of SMZ and SDZ are also considerably lower than the concentration of SMX (for example K'oreje et al. 2016, Chemtai et al. 2023). Maximum concentrations for SMZ in Kenya have been reported as 630 ng/l (K'oreje et al. 2016) and 24 ng/l (Chemtai et al. 2023). In the study by K'oreje et al. (2016), the detection frequency of SMZ was relatively low and it was not detected at most of the sampling points. In a study by Ngigi et al. (2020) maximum concentration for SDZ was reported as 840 ng/l. Many studies have not been investigating the occurrence of SDZ. The measured values for both SMZ and SDZ in Africa are similar to those from Asia. In a study in China by Chen et al. (2014), the maximum concentrations for SMZ and SDZ were reported as 389 ng/l and 113 ng/l, respectively. The concentrations in Africa and Asia are often reported to be higher when compared to values from Europe. For example, in a study by Lopez-Serna et al. (2011) from Spain, the maximum concentrations of SMZ and SDZ were reported as 55 ng/l and 23 ng/l, respectively.

Similar to SMX, TMP is also one of the most frequently and abundantly detected antibiotics in aqueous samples (aus der Beek et al. 2016). In this study, the concentrations of TMP ranged between 36 ng/l to 508 ng/l. Studies on the

occurrence of TMP have been conducted in various regions of Kenya and Africa. Maximum concentrations of TMP in surface water in Kenya have reported as 6950 ng/l (K'oreje et al. 2016), 200 ng/l (Kairigo et al. 2020a), 4400 ng/l (Kairigo et al. 2020b), 2650 ng/l (Ngumba et al. 2016a), 67 (Chemtai et al. 2023), and 3160 ng/l (Ngigi et al. 2020). Most of these reported values are higher than the concentration measured in this study. The concentrations measured from other African countries are generally similar to those from Kenya. For example, a maximum concentration of 2410 ng/l was reported in Zambia (Ngumba et al. 2020), 5875 ng/l in South Africa (Segura et al. 2015), 1374 ng/l in Ghana (Segura et al. 2015), and 6220 ng/l in Mozambique (Segura et al. 2015). The use of TMP is particularly high in many developing countries. As mentioned earlier, TMP is often used in combination with SMX in a drug known as cotrimoxazole, which is utilized in the treatment of HIV/AIDS coinfections (Kairigo et al. 2020a, Ngumba et al. 2020). In cotrimoxazole, the ratio of TMP to SMX is 1:5, leading to lower mass loads and detected concentrations of TMP compared to SMX. (Ngumba et al. 2020) Reported maximum concentrations of TMP in surface water from Europe and Asia are generally lower. For instance, maximum concentrations of 15 ng/l in Finland (Ngumba et al. 2016b), 30 ng/l in Spain (Lopez-Serna et al. 2011), and 128 ng/l in Asia (aus der Beek et al. 2016) have been reported.

In this study, concentrations of CBZ ranged from 17 ng/l to 428 ng/l. K'oreje et al. (2016) reported a maximum concentration of 430 ng/l for CBZ in surface water in Kenya. Higher concentrations have been reported in Africa in a study by aus der Beek et al. (2016) with a maximum concentration of 868 ng/l. In Europe and Asia, the reported maximum concentrations are reported to be for example 118 ng/l in Europe and 26 ng/l in Asia (aus der Beek et al. 2016) The concentrations of CBZ are relatively low compared to for example SMX or TMP. This could be due to lower consumption levels or its less water-soluble properties.

Among tetracyclines, DOX was the most frequently detected, with concentrations ranging from 371 ng/l to 530 ng/l. TET had a detection frequency of 25 % and concentrations ranging from 397 ng/l to 455 ng/l. OXT was not detected in one of the sampling points in this study. Maximum concentrations of DOX in other studies from Kenya have been reported to be 300 ng/l (Kairigo et al. 2020a) and 5 ng/l (Segura et al. 2015), which are lower than the values in this study. In other studies, in Africa, maximum concentrations have been reported as 3260 ng/l in Zambia (Ngumba et al. 2020) and 10 ng/l in Ghana (Segura et al. 2015). TET is detected with significantly higher concentration in many studies from Kenya and Africa, with maximum concentration reported as 434 000 ng/l in Kenya (Segura et al. 2015), 465 000 ng/l in Ghana (Segura et al. 2015), 4220 ng/l in Zambia (Ngumba et al. 2020), and 1290 ng/l in South Africa (Addis et al. 2024). However, low maximum concentrations have also been detected, such as 0.2 ng/l in a study by Chemtai et al. (2023), and <120 ng/l in a study by Ngigi et al. (2020). The occurrence and concentration of OXT have been less studied. A maximum concentration of 60 ng/l in Africa (Segura et al. 2015) has been reported.

The concentrations of tetracyclines in Europe and Asia are also relatively low. In a study by Chen et al. (2014) in China, the maximum concentrations of DOX, TET, and OXT in river water were reported as 112 ng/l, 54 ng/l, and 220 ng/l, respectively. In Spain, the maximum concentrations have been reported as

48 ng/l for DOX, 228 ng/l for TET, and 37 ng/l for OXT (López-Serna et al. 2011). In a study by Ngumba et al. (2016b) in Finland, either TET or DOX were detected in surface water. Tetracyclines are hydrophobic compounds, and they are usually more likely to be absorbed into soil. Thus, they are often detected with lower concentrations from the aqueous phase. (Christian et al. 2003, Addis et al. 2024) Various factors can affect absorption. Tetracyclines can for example form stable complexes with cations, like iron or aluminum. (Felis et al. 2020)

AMX and AMP were detected in some of the samples, with maximum concentrations of 63 ng/l and 32 ng/l, respectively. Other studies in Kenya have reported maximum concentrations of 900 ng/l (Kairigo et al. 2020a) and 2 ng/l (Chemtai et al. 2023) in surface water for AMX. A study by Ngumba et al. (2020) in Zambia reported a maximum concentration of 3410 ng/l, which is significantly higher than concentrations from Kenya. AMP is also detected with relatively low detection frequencies and concentrations in studies in Kenya. Ngigi et al. (2020) and Chemtai et al. (2023) reported maximum concentrations of 240 ng/l and 0.1 ng/l in surface water, respectively. The concentrations of AMX and AMP in Asia and Europe have also been reported to be relatively low. In a study by Li et al. (2018) in China, maximum concentrations for AMX and AMP were reported as 29 ng/l and 18 ng/l, respectively. In a study by Christian et al. (2003) in Germany, the maximum concentration for AMP was reported as 26 ng/l (Christian et al. 2003), whereas AMX was not detected in a study by Ngumba et al. (2016b) in Finland. Due to the beta-lactam ring, AMX and AMP are chemically unstable and easily hydrolyzed, resulting in low concentrations in water samples (Felis et al. 2020, Ngumba et al. 2020, Addis et al. 2024), while concentrations in soil and sediments can be higher. For example, in a study by Kairigo et al. (2020a), higher concentrations of AMX in sediment than in surface water samples were reported.

Both antiretrovirals studied were detected in all samples in this study. Concentrations of 3TC and NVP ranged from 103 ng/l to 3166 ng/l and 58 ng/l to 306 ng/l, respectively. Antiretrovirals have not been studied as extensively as antibiotics, especially studies from developed countries are limited, whereas more studies in sub-Saharan countries have been conducted. This is probably due to the higher usage of antiretrovirals in many sub-Saharan countries for the treatment of HIV/AIDS. Maximum concentrations of 3TC in surface water in Kenya have been reported as 167 000 ng/l (K'oreje et al. 2016), and 5428 ng/l (Ngumba et al. 2016a). High concentrations in Zambia have also been detected in a study by Ngumba et al. (2020), with the maximum concentration reported as 49 700 ng/l. In many studies, the concentrations of NVP are slightly lower than 3TC. Maximum concentrations have been reported as 2300 ng/l (Kairigo et al. 2020b), 4860 ng/l (Ngumba et al. 2016a), 5620 ng/l (K'oreje et al. 2016), and 228 300 ng/l (Kairigo et al. 2020a) in surface water of Kenya. In a study by Ngumba et al. (2020) in Zambia, the maximum concentration was reported as 220 ng/l. The concentrations measured in this study are significantly lower compared to many studies from Africa, but considerably higher than reported values from Europe. For example, in a study by Aminot et al. (2015), maximum concentrations were reported as 4.1 ng/l for 3TC and 1.3 ng/l for NVP. In a study in Finland, 3TC was detected with a maximum concentration of 12 ng/l, while NVP was not detected (Ngumba et al. 2016b).

High concentrations of antibiotics and antiretrovirals, especially in Kenya and other sub-Saharan countries, have been reported in many studies. The concentrations detected in this study were generally lower or similar to reported values from similar regions, but higher than those from some European and Asian countries. As mentioned, numerous factors can impact the presence and fate of pharmaceuticals in the environment. Human activities and consumption levels significantly influence the amount of pharmaceuticals entering the environment. Many studies have, for instance, reported higher concentrations downstream of rivers from the disposal point of WWTP's effluents (for example K'oreje et al. 2016, Kairigo et al. 2020b, Chemtai et al. 2023, Addis et al. 2024). The highest concentrations are usually measured in wastewater effluents, while in rivers the concentrations are lower. This could be due to dilution, phase partitioning, and different degradation processes, such as bio- and photodegradation and hydrolysis, in the environment (Fedaku et al. 2019, K'oreje et al. 2020). Relatively high concentrations have also been reported upstream of rivers and in open drains, which suggests that non-point sources are also significant sources of pollution. In densely populated areas with informal settlements and inadequate sanitation and treatment systems, concentrations are usually higher (Ngumba et al. 2016a, K'oreje et al. 2020). Inadequate wastewater treatment and sanitation are one reason for higher concentrations in many sub-Saharan countries compared to higher-income countries.

Conditions of the environment and seasonality can also have a significant influence (Fedaku et al. 2019, Harrower et al. 2021). In a study conducted by Addis et al. (2024), the differences between seasons were examined. Concentrations were found to be higher during the spring compared to autumn or winter, likely due to varying levels of rainfall between the seasons. Winter typically experiences more rain, leading to a higher dilution of compounds in the river water (Addis et al. 2024). Seasonal variations were also noted in a study by Kairigo et al. (2020b) in Kenya. Samples collected during the dry period (September) contained more residual pharmaceuticals compared to samples from the rainy season (January) (Kairigo et al. 2020b). Significant seasonal variation was also shown in a study by Li et al. (2018). Temperature can also impact the occurrence of compounds, as they are usually more stable in colder temperatures than in higher temperatures (Harrower et al. 2021). In a study by Aminot et al. (2015), concentrations of many pharmaceuticals in colder conditions were shown to be much lower compared to warmer conditions. Additionally, consumption patterns and the most used antibiotics can vary between different regions and seasons (Harrower et al. 2021). In many low-income countries, the use of antibiotics and antiretrovirals is higher and less regulated than in high-income countries, resulting in greater environmental contamination.

The potential for the development of antibiotic resistance was not assessed in most of the studies. In a study by Kairigo et al. (2020a), the risk of antibiotic resistance selection was calculated using the same $PNEC_{(RS)}$ values as in this study. The risks were found to be low to medium for DOX (RQ = 0.1–0.7), low to high for SMX (RQ = 0.1–3.54), and medium to high for AMX (RQ = 0.2–6.4) and TMP (RQ = 0.2–1). Based on the concentrations found in other studies,

particularly those in sub-Saharan countries, there is likely a high risk for resistance selection for many antibiotics. Antibiotic resistance genes and bacteria have been identified in numerous studies, with the number expected only to rise. Further research is needed for a better understanding of the fate and transport of antibiotics and antiretrovirals. Particularly important is research on the formation and presence of resistance genes and bacteria. To ensure that antibiotics can remain effective without negatively impacting the environment or public health, it is also important to utilize more advanced treatment methods for both wastewater and sludge (O'Flaherty & Cummins 2017, Szymanska et al. 2019) Furthermore, it is crucial to implement improved regulations, promote responsible usage of pharmaceuticals and raise awareness of the issues they present.

4.3 Limitations of the study

Some limitations may have affected the results of the study. The selection of sampling points can impact the results. Sampling points were chosen based on Muriuki's study to cover a variety of locations within the study area. However, the sampling points were not the same as those in Muriuki's study due to a lack of precise location information. Additionally, samples from the influents or effluents of the wastewater treatment plant could not be collected as it was no longer operational. Only aqueous samples were collected in this study due to limited time and resources. It would have also been interesting to include for example zidovudine (ZVD) and ciprofloxacin (CIP) in the analysis due to them being also highly used in the area. Despite the careful work, there might have also been errors during sampling and sample preparation, as well as during the analysis part. In the laboratory, the resources were limited at the time. For example, tap water was used in the analyses when Milli-Q water was not available. The recovery values (%) for certain compounds, such as OXT, from the LC-MS/MS runs were relatively low, suggesting potential errors in the working. The risk for antibiotic resistance development was calculated based on measured concentrations. However, the DNA samples were also extracted from the samples, and selected ARGs were analyzed with high-throughput qPCR analysis. Unfortunately, the result from the qPCR analysis could not be included in this thesis.

5 CONCLUSIONS

In this thesis, the presence of commonly used antibiotics and antiretrovirals in the surface water of Juja, Kenya was analyzed, and the risk for antibiotic resistance development was estimated. For the analysis, water samples were collected from the open drains, a river, and a pond in the study area. Two rounds of sampling were conducted during the study. Selected compounds were analyzed using SPE-LC-MS/MS technology. Aims of the study were to measure the concentrations of selected compounds, calculate the risk for resistance

selection based on the measured concentration, and compare the results of this study to previous studies. Concentration varied a lot between compounds and sampling points. Most of the selected compounds were detected in the samples, except for OXT and SDZ. Overall, the measured concentrations of the compounds ranged from 17 ng/l to 4269 ng/l. In both sampling rounds, out of antibiotics, SMX was detected with the highest concentration, with a maximum concentration of 4269 ng/l, while 3TC was the most abundant antiretroviral, with a maximum concentration of 3166 ng/l. The most detected compounds were those commonly used in the area. The concentrations during the second sampling were higher compared to the first sampling, which could be due to seasonal variation and changes in the rainfall amount. Especially the concentrations of SMX were higher during the second sampling, with over five times higher mean concentration. There was also variation among sampling points, which could be due to varying environmental conditions or contamination loads into the environment. Concentrations were higher in densely populated areas, and for example in the open drain near the old WWTP. Generally, concentrations in river samples were lower compared to the open drains, which could be due to higher dilution of compounds to the river. The measured concentrations in this were lower compared to reported values in the previous studies in the area and other sub-Saharan countries, but higher than in many higher-income countries. The risk for resistance selection varied from low to high, with TMP having the highest risk for resistance selection. Antimicrobial resistance poses a significant threat to human health and endangers the achievement of many SDGs. Therefore, it is crucial to consistently evaluate and quantify the presence of antibiotics and antiretrovirals, as well as the resistance genes and bacteria, in the environment to reduce their impact on both the environment and human health.

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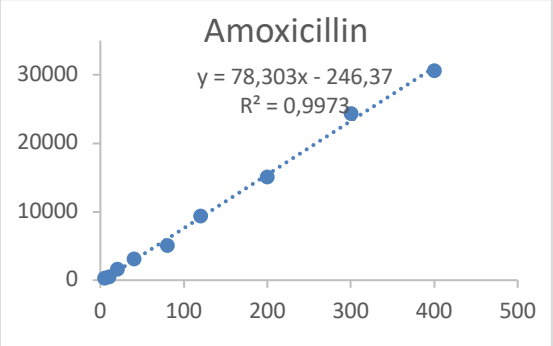
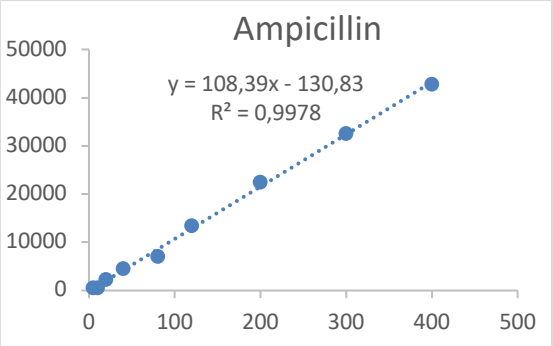
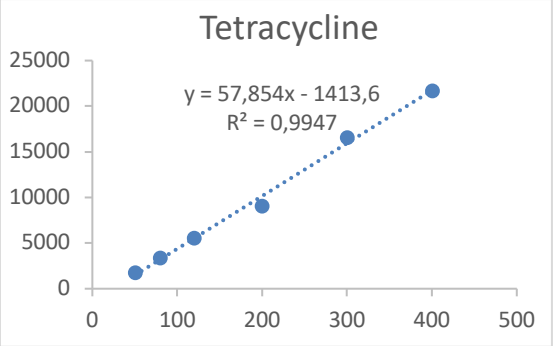
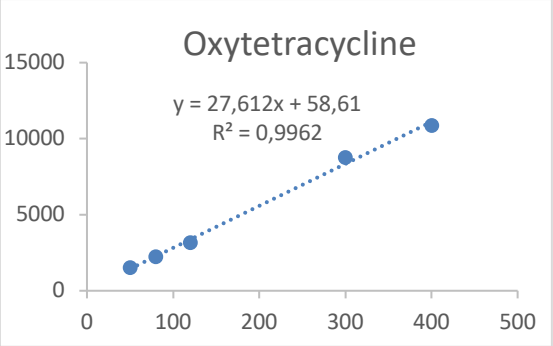
APPENDIX 1. CALIBRATION CURVES

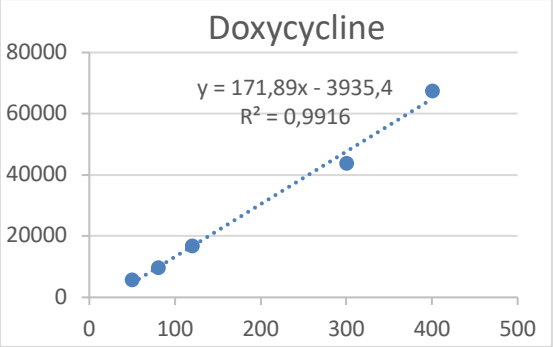
LC-MS/MS calibration curves for selected antibiotics and antiretroviral drugs. In the y-axel is the analyte area and, in the x-axel is the concentration of the analyte (µg/l).

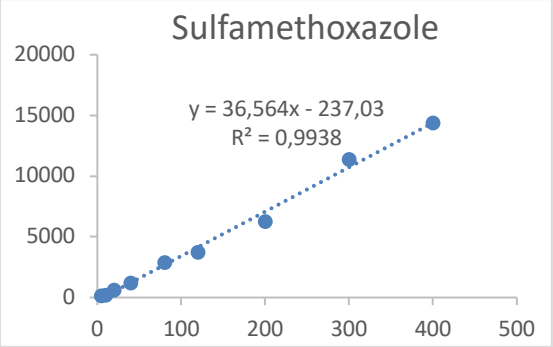


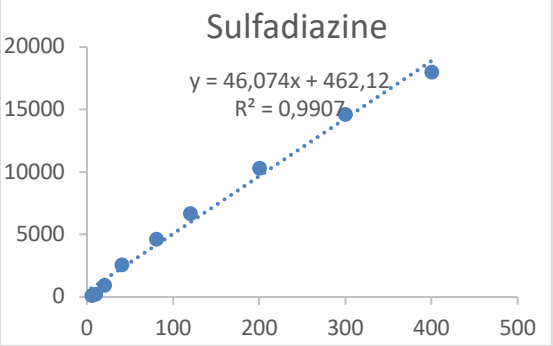


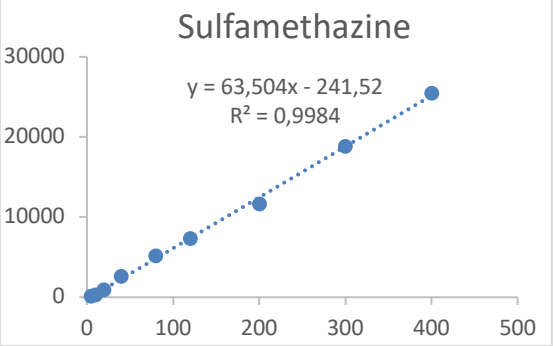


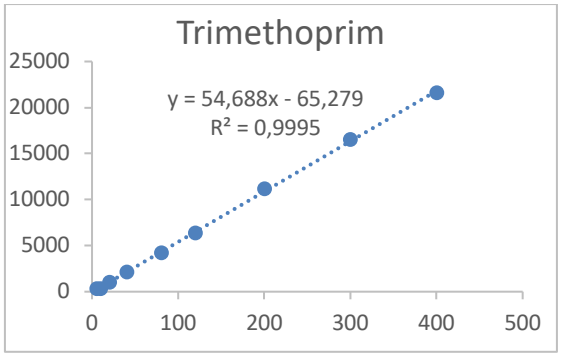


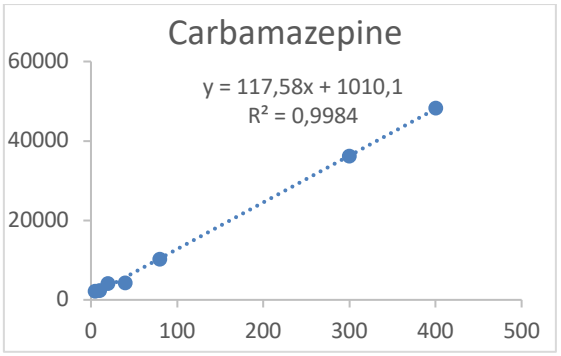


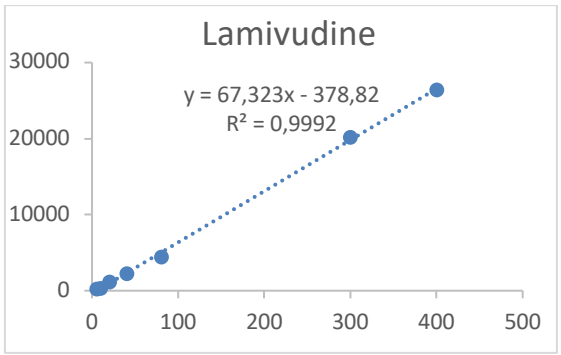


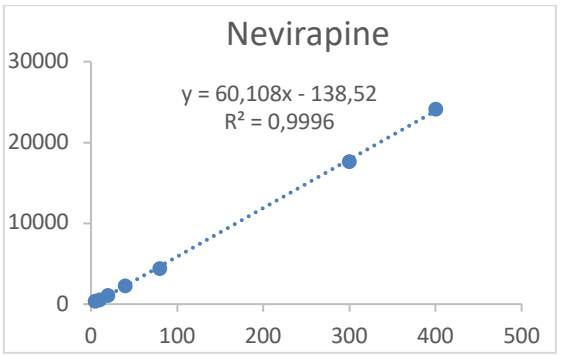





Trimethoprim
y = 54,688x - 65,279
R² = 0,9995
25000
20000
15000
10000
5000
0
0
100
200
300
400
500


Carbamazepine
y = 117,58x + 1010,1
R² = 0,9984
60000
40000
20000
0
0
100
200
300
400
500


Lamivudine
y = 67,323x - 378,82
R² = 0,9992
30000
20000
10000
0
0
100
200
300
400
500


Nevirapine
y = 60,108x - 138,52
R² = 0,9996
30000
20000
10000
0
0
100
200
300
400
500