



**INVESTIGATION OF CONTAMINATION OF COMMUNITY GROUNDWATER  
SOURCES WITH ANTIBIOTICS IN INFORMAL SETTLEMENTS OF KISUMU,  
KENYA**

By  
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PHILOSOPHY**

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**2023**

## DECLARATION

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I, Kellen Joyce Karimi, declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, and has not been submitted for examination before for any degree or examination at any other University.



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(Signature of candidate)

\_\_\_\_ 25<sup>th</sup> \_\_\_\_\_ day of \_\_\_\_\_ September \_\_\_\_\_ 2023 \_\_\_\_\_ at \_\_\_\_\_ 10.00 AM \_\_\_\_\_

## **Dedication**

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To Vincent, my husband for being my greatest supporter and our sons Oliver and Leon for persevering the many instances when mom was away.

## **Presentations arising from this research project**

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**Karimi J. K**, Aijaz A, Duse A. G, Olago D, Ndwigah S. N., Mwanthi A.M, Ayah R, Dulo S. Investigation of contamination of groundwater sources with antibiotics in informal settlements of Kenya. *Stakeholder dissemination workshop Kisumu, Kenya, 29<sup>th</sup> March 2022.*

**Karimi J. K**, Ngumba E, Aijaz A, Duse A. G, Olago D, Ndwigah S. N, Mwanthi A.M, Ayah R, Dulo S. Groundwater contamination with Sulfamethoxazole and Trimethoprim and spread of drug resistant *E. coli* in informal settlements; Kenya. *UNESCO- IWRA (United Nations Educational, Scientific, and Cultural Organization, International Water Resources Association) Online conference 17-19<sup>th</sup> January 2023. (Emerging pollutants: Protecting Water Quality for the Health of People and the Environment.*

## Publications arising from this research

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**Karimi, K.J.**, Ahmad, A., Duse, A., Mwanthi, M., Ayah, R. Prevalence of Antibiotic Use and Disposal at Household Level in Informal Settlements of Kisumu, Kenya. *Int. J. Environ. Res. Public Health* 2023, 20, 287. [https:// doi.org/10.3390/ijerph20010287](https://doi.org/10.3390/ijerph20010287) (Appendices-Publication 1)

**Karimi K.J.**, Ngumba E, Aijaz A, Duse A.G, Olago D, Ndwigah S.N., Mwanthi A.M, Ayah R, Dulo S. Contamination of groundwater with sulfamethoxazole and antibiotic resistant *Escherichia coli* in informal settlements in Kisumu, Kenya. *PLOS Water* 2023, DOI: [10.1371/journal.pwat.0000076](https://doi.org/10.1371/journal.pwat.0000076) (Appendices- Publication 2)

## **ABSTRACT**

Antibiotics have been used to cure diseases, but there are growing concerns about the risk to human health caused by inadvertent exposure to low levels of antibiotics in the environment. Despite extensive reporting from the developed world on antibiotic pollution of groundwater, relatively little study has been conducted on antibiotic contamination of groundwater in the developing countries, particularly informal settlements. Antibiotic usage and misuse have long been seen as clinical events, with little understanding of the role of disposal in the development of environmentally induced resistance. Exposure pathways that contribute to groundwater contamination in informal settlements put residents at odds because they already face inequalities, such as a high disease burden exacerbated by antibiotic resistance; thus, proper antibiotic disposal is critical in protecting human and environmental health. The purpose of this cross-sectional study was to establish the prevalence of groundwater contamination with the common antibiotics' such as sulfamethoxazole, trimethoprim, and metronidazole, and the related antibiotic resistance and the human health risk of exposure. Ethical clearance to conduct research was obtained from three institutions as follows: - the Health Research Ethic Committee of the university of the Witwatersrand (HREC. Protocol Number M190412); the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UoN-ERC. Ref No. P71910/2018); and the National Commission for Science, Technology, and Innovation (Ref No. NACOSTI/P/19/3232/28732). Each respondent gave informed consent to participate in the study. Anonymity was maintained at all levels of the study to protect the study participants from identification. Antibiotic use, which is connected to antibiotic disposal, was evaluated in a random sample of 447 families. From the 188 mapped groundwater sources, a random sample of 49 groundwater sources was chosen, and water samples were taken for antibiotic concentration analysis utilizing a solid-phase extraction and liquid chromatography coupled to magnetic sector high resolution mass spectrometry (SPE-LC-MS/MS). The Kirby-Bauber diffusion method was used to test antibiotic resistance in *Escherichia coli*. The community's potential groundwater contamination routes were assessed by determining antibiotic use and disposal among households as well as assessing the environmental risk of exposure. In the households visited, 75% (n=337) were female and 25% (n=110) were male. The prevalence of antibiotic use in informal settlements was 43% (n=193), with 70% (n=137) users reporting that they obtained the antibiotics through a prescription from a health practitioner. A significant relationship was observed between having

HIV/AIDS and acquiring antibiotics through a prescription;  $p=0.001$ . An association was also observed among the informal settlements, where a lower number of MNY B dwellers did not receive a prescription for the antibiotics acquired. There was no statistically significant difference in antibiotic use between males and females; odds ratio=1.33; whereas there was a difference in HIV/AIDS status; odds ratio=0.313; and among informal settlements where the odds of using antibiotics were reduced in NY B; odds ratio=0.42.

Respondents who used antibiotics either kept the unused antibiotics for future use 87.1% ( $n=27$ ) or disposed them. Among the disposals 51.6% ( $n=16$ ) disposed in pit latrines, 16.1% ( $n=5$ ) dispose in compost pits, and 6.5% ( $n=2$ ) dispose the remaining antibiotics by burning. Females completed their antibiotic doses at a higher rate (36.3%;  $n=117$ ) than males (32.5%;  $n=39$ ). Significant difference was observed in completion rate among the HIV/AIDS positive and negative respondents as well as among informal settlements;  $p<0.000$  and  $p=0.001$  respectively. On the other hand, groundwater use in these communities is widespread. Respondents used it for a variety of purposes, including drinking (9%;  $n=39$ ), though they declined to report. Awareness of the health consequences of drinking antibiotic-contaminated water was also low (35%;  $n=158$ ), especially among households that reported antibiotic use;  $p=0.003$ .

Only Sulfamethoxazole was detected in 7 out of 49 groundwater samples at a detection frequency of 14.3%; with concentrations ranging from *nd* to 258 ng/L. *Escherichia coli* and *Cryptosporidium parvum* were isolated from all the 49 water samples and *E. coli* isolates from 3 (6%) water samples were resistant to sulfamethoxazole with Inhibition Zone Diameters of 0.8 mm, 10.5 mm, and 11.5 mm. The 3 water samples were however not among samples where sulfamethoxazole was detected. The Hazard Quotient was 0 (zero), and therefore no risk of exposure to sulfamethoxazole in the environment, but the level of antibiotics that trigger antibiotic resistance is not known. Because of the rising problem of antibiotic resistance due to overuse and incorrect disposal, teaching on safe antibiotic prescription should be incorporated into medical training for all cadres. In addition to educating patients on proper use and disposal, the ministries of health should ensure the antimicrobial stewardship standards are adhered to both locally and worldwide. Follow-up research of antibiotic resistance discovered in three groundwater sources must be done to eliminate the possible sources and prevent further spread. This study is instrumental in informing the inclusion of antibiotics on the list of frequently monitored contaminants during water treatment, as well as serving as a starting point for antibiotic surveillance in Kenya.

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## **DEFINITION OF TERMS**

### **Groundwater**

Groundwater is a collection of water below the surface in rocks and soils, that accumulates in aquifers (WHO, 2006).

### **Emerging contaminants**

Emerging contaminants (EC's) are a group of compounds recently detected in the environment which include water. The contaminants include personal care products (PCP's) and pharmaceutical products

### **Antibiotics**

These are medicines that fight different types of bacterial infections in human being and animals. The antibiotics work by killing or inhibiting growth of the bacteria.

### **Antibiotic resistance**

This is the ability of microbes to resist the effects of drugs. The microbes are either not killed or their growth is not inhibited by the drugs.

### **Human health risk assesment (HHRA)**

This is the process intended to estimate the nature and probability of adverse health effects to a target organism (humans) or a section of a population as a result of exposure to a particular agent or hazard contaminating the environmental media now and in the future (WHO, 2004).

### **Informal settlements**

This is synonymous with slums or unplanned settlements and are residential areas where a group of housing units has been constructed and not in compliance with current planning and building regulations (Un-Habitat, 2003).

## Abbreviations and acronyms

|        |                                            |
|--------|--------------------------------------------|
| AIDS   | Acquired Immune Deficiency Syndrome        |
| API's  | Active Pharmaceutical Ingredients          |
| ARB    | Antibiotic Resistant Bacteria              |
| ARG    | Antibiotic Resistant Genes                 |
| ARV    | Antiretroviral                             |
| BW     | Body Weight                                |
| CD4    | Cluster of Differentiation 4               |
| CDC    | Centre for Disease Control                 |
| CFD    | Centrifugation Filtrate Device             |
| CR     | Contact Rate                               |
| DIC    | Differential Inference Contrast            |
| DNA    | Deoxyribonucleic Acid                      |
| ECOSAR | Ecological Structure Activity Relationship |
| ED     | Exposure Duration                          |
| EDTA   | Ethylene diamine tetra acetic acid         |
| EF     | Exposure Frequency                         |
| EOC    | Emerging Organic Contaminants              |
| EOC    | Emerging Organic Contaminants              |
| EPA    | Environmental Protection Agency            |
| FITC   | Fluorescein Isothiocyanate                 |
| GIS    | Geographical Information System            |
| GoK    | Government of Kenya                        |
| HHRA   | Human Health Risk                          |
| HPLC   | High Performance Liquid Chromatography     |
| HQ     | Hazard Quotient                            |
| ICM    | Iodate Contrast Media                      |
| IZD    | Inhibition Zone Diameter                   |
| KEBS   | Kenya Bureau of Standards                  |
| LMICs  | Low-and-Medium-Income Countries            |

|                  |                                                                                |
|------------------|--------------------------------------------------------------------------------|
| LOD              | Limit of Detection                                                             |
| LOQ              | Limit of Quantitation                                                          |
| MEC              | Measured Environmental Concentration                                           |
| MIC              | Minimum Inhibition Concentrations                                              |
| MoH              | Ministry of Health                                                             |
| MPH              | Master of Public Health                                                        |
| MTZ              | Metronidazole                                                                  |
| NACOSTI          | National Commission for Science and Technology                                 |
| NHLS             | National Health Laboratory Services                                            |
| NOAEL            | No-Observable Adverse Effect Level                                             |
| NSAIDs           | Nonsteroidal Anti-inflammatory Drugs                                           |
| PCR              | Polymerase Chain Reaction                                                      |
| PDE-V            | Phosphodiesterase 5                                                            |
| PEC              | Predicted Environmental Concentration                                          |
| pH               | Potential Hydrogen                                                             |
| PNEC             | Predicted No Effect Concentration                                              |
| RfD              | Reference Dose Factor                                                          |
| RQ               | Risk Quotient                                                                  |
| RR LC/MS         | Rapid Resolution Liquid Chromatography coupled with a tandem mass spectrometer |
| SDG              | Sustainable Development Goals                                                  |
| SMX              | Sulfamethoxazole                                                               |
| SPE              | Solid Phase Extraction                                                         |
| SPE-LC-ESI-MS/MS | Solid Phase Extraction -Liquid Chromatography tandem Mass Spectrometry         |
| TB               | Tuberculosis                                                                   |
| TMP              | Trimethoprim                                                                   |
| UF               | Uncertainty Factor (UF)                                                        |
| UN               | United Nations                                                                 |
| USA              | United States of America                                                       |
| UTI              | Urinary Tract Infection                                                        |
| WHO              | World Health Organization                                                      |

**CHAPTER ONE:****INTRODUCTION****1.1 Background**

The discovery of antibiotics in the 20<sup>th</sup> century was considered as one of the revolutionary development that can serve a solution to infectious diseases caused by various pathogens in humans and animals (Carvalho & Santos, 2016; Roose-Amsaleg & Laverman, 2016). Recently antibiotics have been classified as an emerging source of environmental contamination since their presence in almost all environmental media raises concerns of their effects on non-target organisms in the environment (Roose-Amsaleg & Laverman, 2016). The effects include antimicrobial resistance and development of antibiotic resistant genes (ARG) as well as environmental toxicity (Carvalho & Santos, 2016; Pan & Chu, 2018).

The antibiotics that have been detected in water over time in different ecosystems are the same antibiotics recommended for use by humans to prevent or cure diseases as well as in veterinary medicine for disease prophylaxis and growth promotion (Milić *et al.*, 2017). These antibiotics end up in the environment and groundwater through a cycle of cross-contamination, as explained in *figure 1* (Daughton G, 2008; Lamastra *et al.*, 2016; Milić *et al.*, 2017). Points of accumulation of the antibiotics that end up contaminating groundwater have been identified to be health care facilities waste disposal, wastewater used for irrigation, sewage, landfills, animal waste and pit latrines (Azanu *et al.*, 2018; Mceachran *et al.*, 2017; Oskam *et al.*, 2021).

**1.2 Groundwater**

Groundwater comprises of boreholes, shallow wells and natural springs, which are naturally occurring and easily accessible where the aquifer is shallow and at the same time prone to pollution as a result of natural and human activities (Lamastra *et al.*, 2016; Zaporozec *et al.*, 2002). With the phenomenon of global warming, and predicted increase in population growth, there is demand for water and groundwater is slowly becoming scarce, especially in informal settlements where people cannot afford piped water (United Nations, 2015). For this reason, groundwater should therefore be protected. Antibiotics being an emerging form of groundwater contaminant must be monitored and impact on environment and water sources determined by reviewing the pathways to contamination.

### 1.3 Pathways to groundwater contamination

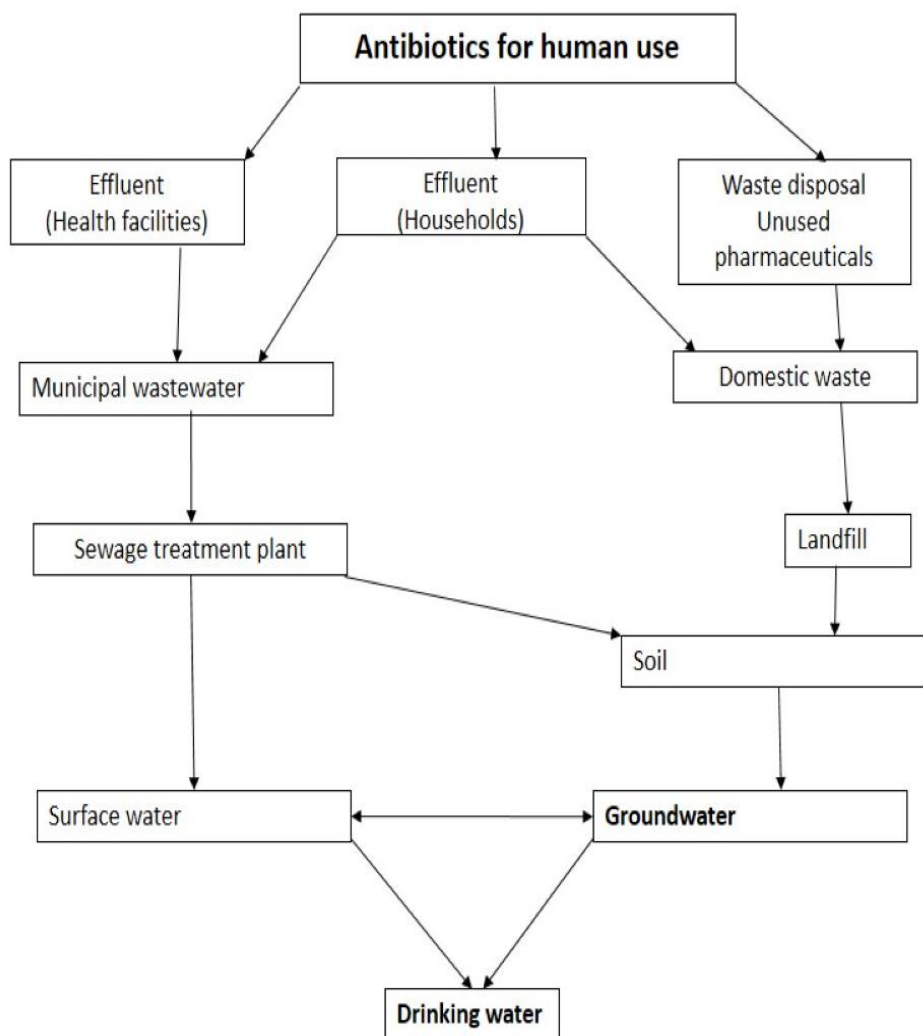


Figure 1: Pathways to exposure to groundwater contamination to humans

(Source: (Milić *et al.*, 2017)).

In the recent past, the knowledge of contamination of the environment and water sources with these emerging contaminants has continued to increase and the concentration of such contaminants deserve attention (Petrie *et al.*, 2016). This has necessitated calls for more research to better understand occurrence, fate and ecological impact, including human health effect resulting from chronic exposure to organic contaminants (Sorensen *et al.*, 2015). Studies have mostly focused

on the occurrence of pharmaceutical products in surface water (Hughes *et al.*, 2013) with little on groundwater (Lapworth *et al.*, 2012; Sui *et al.*, 2015).

Sources of groundwater pollution have been identified, as shown in *figure 1*, and include waste disposed by health care facilities (Mendoza *et al.*, 2015) and effluent from pharmaceutical industries. Furthermore, cross-contamination of the water sources by nearby sewage sanitation facilities, such as pit latrines or leaky sewer lines that may seep into groundwater (Graham & Polizzotto, 2013; Kimani-Murage & Ngindu, 2007). Little is known about the community level environmental contamination cycle. On the other hand, communities are not aware of proper use of antibiotics nor are they aware of antibiotic resistance and the effects of resistance (Yusef *et al.*, 2018). Incidentally, effects of exposure to organic contaminants will continue to be a threat, since they are not completely removed from wastewater during treatment, making wastewater a major source of environmental exposure in most countries (Fick *et al.*, 2009; Koopaei & Abdollahi, 2017).

#### **1.4 Pharmaceutical contaminants in groundwater**

Contamination of water sources with pharmaceutical compounds and residues has been determined in both surface water and to some extent in groundwater. Antibiotics have been found to be among pharmaceuticals compounds detected in groundwater among others that include, antidepressants, antiretroviral drugs, antimalarial drugs, anti-inflammatory drugs, antihypertensive medication among others (Fabbri, 2015; Guerra *et al.*, 2014; Ngumba *et al.*, 2016). Different antibiotics belonging to different classes have been detected in groundwater which include sulfamethoxazole, trimethoprim and metronidazole (K'oreje *et al.*, 2016; Ngumba *et al.*, 2020). These three antibiotics are important for the reduction of burden of disease among the HIV as they are used for prophylaxis against opportunistic infections. The prevalence of HIV in the study area has been reported to be 16.3%, higher than the national level of 4.9%.

#### **1.5 Antibiotics contaminants in groundwater**

##### **1.5.1 Sulfamethoxazole**

Sulfamethoxazole is an old antibiotic in the class of Sulfonamides, formulated in combination with trimethoprim in 1969 (Straub, 2016). Sulfamethoxazole act as a Cytochrome P450 2C9 inhibitor which completely inhibits dihydropteroate synthase, thus inhibiting the production of dihydropteroic acid required for the formation of folic acid, which is necessary for bacterial growth. The antibiotic has been detected in environmental media in Europe over the years, dating

back to as early as 1985. The predicted no effect concentration, PNEC indicated no risk among humans due to indirect exposure in the environment risk but showed risk among aquatic organisms, therefore, recommends conducting of risk assessments in other parts of the continent.

### Properties of sulfamethoxazole

Sulfamethoxazole is an odourless yellow-white powder that is bitter in taste. Sulfamethoxazole structural analogue of para-aminobenzoic acid (*Figure 2*). It is barely soluble in solvents like water, ether and chloroform and a melting point of 167°C. Sulfamethoxazole is slightly soluble in water (0.5g/L) (WHO, 2001).

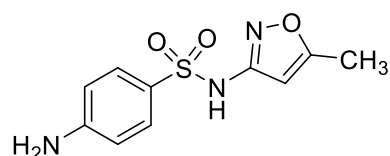


Figure 2: Chemical structure of sulfamethoxazole

### 1.5.2 Trimethoprim

Trimethoprim is a pyrimidine inhibitor of dihydrofolate reductase. It is mostly used in combination with sulfamethoxazole. Trimethoprim is sometimes used alone or in combination with sulfamethoprim in treatment of malaria (Gaillard *et al.*, 2016).

Trimethoprim is a white-cream crystalline powder. It is odourless and has a bitter taste. The compound is slightly soluble in water and alcohol, but solubility is dependent on the pH of the solvent. Trimethoprim is a weak base and does not dissolve in solvents with high pH. The chemical structure of trimethoprim is as shown in (*Figure 3*).

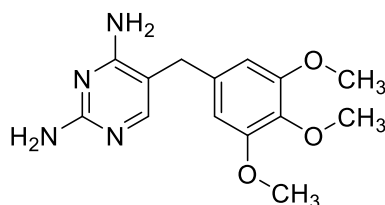


Figure 3: Chemical structure of trimethoprim

### Sulfamethoxazole- trimethoprim

The combination of Trimethoprim and Sulfamethoxazole is used to treat various infections like urinary tract infections, respiratory tract infections, traveller's diarrhoea and also for prophylaxis among patients with compromised immunity in the treatment of urinary tract infection (UTI) (Crellin *et al.*, 2018; Demarsh *et al.*, 2020) and Pneumocystis (carinii) jiroveci pneumonia (Crellin *et al.*, 2017).

Stability and poor solubility in water of the two compounds is a clear indication that they remain unchanged in water gives them the contaminant characteristics (Pourmokhtar & Jacobson, 2005). In addition, they are likely to result in undesirable effects like leading to development of antibiotic resistance. Bacterial resistance to both trimethoprim and sulfamethoxazole has been reported in urinary tract infections in different settings, suggesting the need for review of antibiotics (Gangcuangco *et al.*, 2015; Guneyssel, 2016).

### 1.5.3 Metronidazole

Metronidazole is a synthetic nitromidazole known to have antimicrobial and antiparasitic properties. Metronidazole is known to treat parasitic infections like cryptosporidium, amebiasis, vaginitis, trichomonas, giardiasis, and anaerobic bacteria, like clostridia (Kolodzieska M, Maszkowska J, Bialk-Bielinska A, Stepnowski P and Kumirska, 2013). The mechanism of action is not well understood, but after ingestion the drug is known to inhibit deoxynucleic acid (DNA) synthesis by causing strands breakage which prevents bacterial cell growth. Metronidazole appears as cream-coloured crystals that are odourless and does not degrade in water.

Metronidazole is excreted unchanged after ingestion, leading to accumulation in water and difficult to remove during wastewater treatment making it an environmental pollutant (Nolasco *et al.*, 2013).

The chemical structure of metronidazole is as shown in *figure 4*.

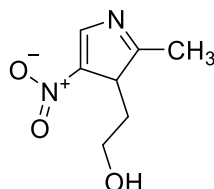


**Figure 4: Chemical structure of metronidazole**

Microbial resistance to metronidazole has been determined in vitro among different organisms that include *bacteroides fragilis*, *Helicobacter pylori*, *Trichomonas*, *Clostridium difficile*, *Entamoeba* and *giardia* (Dhand & Snyderman, 2009).

## 1.6 Problem statement

Health effects of chronic exposure to low concentrations of pharmaceutical products in the environment, particularly emerging organic contaminants have been found to be harmful, including causing endocrine disruption in aquatic animals (Corbel *et al.*, 2009; Kusturica & Jevtic, 2022). Various antibiotics used by human beings to treat infections have been increasingly detected in the environments (Meng *et al.*, 2019), but their presence in water and the role they play in involuntary exposure in human beings is not well understood (Wang *et al.*, 2016). It is for this reason that it is feared that existence of antibiotics in the environment may pose the threat of antibiotic resistance and harbouring antibiotic resistant bacteria (ARB) (Martínez, 2008; Xi *et al.*, 2009) and this is a public health concern globally.

Concerns over potential adverse effects are growing since the emerging contaminants have been found to move from adjacent points of concentration to the groundwater sources leading to pollution. The concentration points for these contaminants have been found to be the modern day sanitation solutions which include pit latrines, landfills, disposal sites, wastewater treatment sites and inappropriate disposal of the antibiotics (Chen *et al.*, 2017; Dasgupta & Sengupta, 2015; Graham & Polizzotto, 2013; Kimani-Murage & Ngindu, 2007; Matongo *et al.*, 2015; Ngumba *et al.*, 2020).

Investigating the concentration of antibiotics in diverse environmental media at which resistance is developed is imperative, (Berglund, 2015) as a way of mapping the hotspots for antibiotic resistance. Sulfamethoxazole, trimethoprim and metronidazole antibiotics, used in the treatment of opportunistic infections like urinary tract infection and parasitic infections among HIV/AIDS patients have been detected in water sources in an informal settlement in Kisumu (K'oreje *et al.*, 2016). Furthermore, the effect of chronic exposure to antibiotics on human has not been extensively studied (Carvalho & Santos, 2016), especially to vulnerable populations, like people infected with the Human Immunodeficiency Virus (HIV) patients.

The study site is in a region of Kenya that suffer a high burden of HIV/AIDS with the adult prevalence in Kisumu county at 16.3%, which is ranked 3<sup>rd</sup> in the county and more than three times higher than the national prevalence of 4.9% (NACC, 2018) (MOH, 2016). This may explain the occurrence of antibiotics sulfamethoxazole, trimethoprim and metronidazole (K'oreje, Vergeynst, Ombaka, De Wispelaere, *et al.*, 2016). The study area being in informal settlements, the concentrations may be high owing the fact that population density is considerably higher (Ngumba *et al.*, 2015). Presence of the antibiotics in groundwater is a public health risk which manifest as antibiotic resistance to this immunocompromised population and a looming threat to global health and development in the 21st century (Denegre *et al.*, 2019).

Antibiotic resistance has always been regarded as a clinical phenomenon, but recently non-clinical sources of antibiotic resistance have been documented in the environment which include water (Berglund, 2015). Microbes causing opportunistic infections have been reported in clinical settings to be resistance to sulfamethoxazole and trimethoprim among HIV positive paediatric patients in Lima, Peru (Romero *et al.*, 2010). In the treatment of fecal *Escherichia coli* among adults HIV infected patients, initiation of sulfamethoxazole and trimethoprim leads and cross-resistance to other antimicrobials (Egwuatu *et al.*, 2016). In addition to long tenacity and bioaccumulation of the antibiotics in the environment that lead to development of antibiotic resistance, there has also been concerns of risks of toxicity, necessitating more research (Ebele *et al.*, 2017; Serweci'nska, 2020; Mendoza *et al.*, 2015; Ricart *et al.*, 2010). Sulfamethoxazole and trimethoprim are stable in water and barely degrade due to their compact structures, therefore persist in the environment leading to toxicity which is a public health hazard (Yu *et al.*, 2017).

On the other hand, informal settlements have been on the increase, which translate to increased population relying on groundwater sources as a source of domestic water due to shortages (Karanja, 2010) occasioned by scarcity and unreliability of piped water network provided by the Kisumu County government (Okotto *et al.*, 2015). Groundwater is made easily accessible by the fact that the aquifer (water table) in the region is shallow, which is a proxy to ease of contamination.

It is known that most water related health effects have been linked to poor sanitation facilities, characterized by high levels of helminthic infections and coliforms infections (Lapworth *et al.*, 2012; Shayo *et al.*, 2023). Besides, there is also needed to consider other risks due to contaminants found in water that could contribute to negative health outcomes that are not necessarily related to sanitation like antibiotic resistance, a situation where response to treatment is diminished as a result

of development of resistance by target disease causing pathogens. It is important to consider that Kenya is among the countries of the world that does not report on antibiotic surveillance on antibiotic use and antibiotic resistance (World Health Organization, 2014). Nonetheless, in the countries that report antibiotic surveillance, resistance has been reported among bacteria to a given range of antibiotics that include sulfamethoxazole, trimethoprim or metronidazole (World Health Organization, 2014). This notwithstanding, organisms have been found to develop resistance towards the antibiotics, with trimethoprim and sulfamethoxazole reported to have the highest resistance levels towards *Escherichia coli* (Guneysel, 2016).

A study on occurrence of pharmaceuticals in multiple sites in Nairobi and Kisumu that included one shallow well in the study site found among other pharmaceutical compounds, antibiotics sulfamethoxazole, trimethoprim, and metronidazole in surface water. The survey only studied water from one groundwater source which may not be generalized to rest of the informal settlements in the entire Kisumu County. This is with the knowledge that opportunistic infections are known to be the most common course of death among people suffering from HIV/AIDS (Huang *et al.*, 2013). The prevalence of infections like UTI among HIV patients has therefore been determined and emphasis put on focusing on treating urinary tract infections among the patients (Skrzat-klapaczy *et al.*, 2018) and chronic diarrhoeal diseases treatable with the antibiotics (CDC, 2022).

Presence of antibiotics in aquatic media and groundwater have been studied, but the effect of low-level chronic exposure is barely known (Janecko *et al.*, 2016); far less is known on effect on human beings. Human health risk associated with exposure to antibiotics in the environment have been characterized in aquatic environments, but the actual health effects have not been quantified (Koopaei *et al.*, 2017), therefore the public health importance of antibiotic contamination of groundwater sources should not be underestimated.

Environmental risk assessment of exposure to antibiotics among aquatic life has been based on use of antibiotics, with risk being found to be low (Iatrou *et al.*, 2014). However, human health risk assessment of exposure to antibiotics and possible development of antibiotic resistance among vulnerable populations has not been determined (Ashbolt *et al.*, 2013; Serwecińska, 2020), more so exposure to antibiotics via drinking water. Hazard Quotient (Mendoza *et al.*, 2015), has been found to be a measure of assessing risk among humans computed for non-carcinogenic compounds. These are substances that are tolerated since the body can recover from exposure; and antibiotics can be considered part of these substances.

There exists a gap in knowledge on the prevalence of contamination of groundwater sources in informal settlements where water is scarce. The contribution of contamination of groundwater sources with antibiotics in the development of environmentally acquired antibiotic resistance is also lacking especially among microbes that lead to development of opportunistic infections especially among people with compromised immunity like HIV/AIDS patients.

This study, therefore, investigated the concentrations of three antibiotics, determined their prevalence of contamination of groundwater sources in five informal settlements of Kisumu County, Kenya where the water table (aquifer) is shallow and easily accessible. The study assessed routes of contamination with antibiotics in the community by determining antibiotic use and disposal among households. Presence of antibiotic resistance was also determined, as well as risk assessment because of exposure to the antibiotics in water. The study contributes towards facilitation of inclusion of antibiotics in the list of contaminants routinely detected, monitored and removed during water treatment which is an important element of management of water quality as well as protection of this natural resource. The results of the study thus form a basis for the surveillance for antibiotic use to mitigate against antimicrobial resistance as a result of environmental contamination in study area, Kenya and globally.

## **1.7 Objectives**

### **1.7.1 Study objective**

The main objective of the study was to investigate the extent of contamination of community groundwater with sulfamethoxazole, trimethoprim and metronidazole in Kisumu County, Kenya.

### **1.7.2 Specific objectives**

1. To assess sources of antibiotic contaminants in community groundwater in five informal settlements in Kisumu, Kenya
2. To determine and compare the concentration levels of sulfamethoxazole, trimethoprim, and metronidazole in all groundwater sources these informal settlements in Kisumu, Kenya
3. To determine the presence of antibiotic resistant bacteria in water isolated from groundwater sources in the five informal settlements in Kisumu, Kenya
4. To assess human health risk of exposure to antibiotics in the environment in the selected informal settlements in Kisumu, Kenya

## 1.8 Conceptual framework

The figure below outlines the conceptual framework showing the contamination of groundwater sources with emerging pollutant (antibiotics) leading to risk of exposure in the environment and antibiotic resistance in the form of antibiotic resistant bacteria (ARB) or antibiotic resistant genes (ARG).

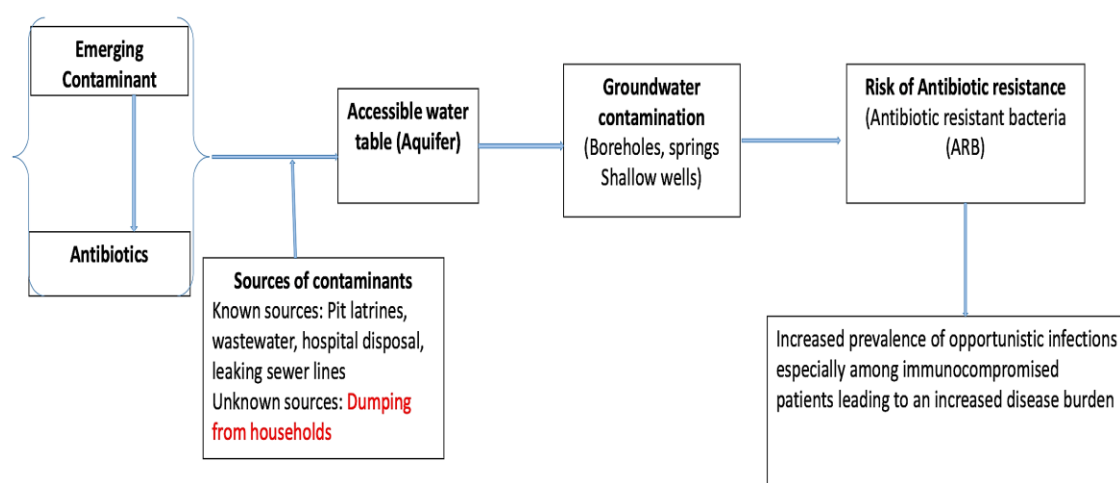


**Figure 5: Conceptual framework showing paths leading to contamination of groundwater and risk antibiotic resistance.**

The environment is considered a health system (Briggs, 2008), with an environment media of interest here being groundwater. Projected population growth (UNDESA, 2015) effects of climate change as a result of global warming is a threat to natural resources like groundwater. The resource is prone to contamination from both known and unknown sources of emerging contaminants like antibiotics. Pathways that link antibiotics for both human and veterinary use to groundwater contamination have been developed, despite its importance in domestic use and agriculture.

Contamination of groundwater sources is linked with proximity to known points of contamination as well as antibiotic use and disposal of uncompleted doses. The effect of the antibiotic contaminants has been linked to development of antibiotic resistance in bacteria that affect human beings and other effects like toxicity to aquatic life. Studies showing the relationship between proximity to points of contamination versus the levels of antibiotic resistance are limited. The

effect of involuntary environmental exposure to antibiotics have been described showing no effect, but effect of chronic exposure has not been determined. The ultimate outcome of exposure to antibiotics in groundwater would be increased prevalence of opportunistic infections, especially among patients with suppressed immunity for example HIV/AIDS patients.

Groundwater, as a naturally occurring resource, should be protected from contamination of any form and it is imperative that studies pertaining to contamination of the sources by antibiotics are conducted in view of the impact of pharmaceutical contaminants on the environment and water sources.

This framework incorporates community ‘participation’ in groundwater contamination in the communities where the groundwater is relied upon as a source of water for domestic use.

A holistic approach towards the achievement of the global Sustainable Development Goals (SDG) is imperative as all components of the SDG do not exist in isolation. This brings in the facet of assimilating the effect on contamination of groundwater sources and the health risks associated with exposure to be able to determine impact on health, especially of vulnerable populations. The current framework therefore proposes to answer the research question; *‘Is contamination of groundwater sources with antibiotics an emerging health threat?’*

## 1.9 Justification

People living in informal settlement of Kisumu largely depend on groundwater for domestic use to supplement surface water supply by the county government which is not reliable due to shortages (Okotto J *et al.*, 2015). These fresh water shortages may be attributed to increased urbanization and a projected population growth, coupled with effects of climate change (Okello, *et al.*, 2015). Groundwater is therefore an important resource to enhance water provision for the growing population habiting the informal settlements.

Pharmaceutical products have been continuously detected in the environment and aquatic media which include groundwater (Ngumba, *et al.*, 2020). The pharmaceutical residues detected are used by human beings as well as veterinary for prophylaxis and treatment of infections (Nguyen *et al.*, 2015; Pan & Chu, 2018). Various studies on occurrence on the pharmaceutical compounds have termed detection levels as trace, but there remain concerns of effects of involuntary chronic exposure. In addition, the contaminants are continually released in the environment resulting into

a constant source of contamination, leading to increased research to understand the fate and effect of the pharmaceutical residues (Petrie et al., 2016; Sorensen et al., 2015).

Antibiotic use has increased globally due to increased demand, especially in developing countries (Gelband & Miller-petrie, 2015). This has caused concern about the negative impacts on the environment and harm to the health humans and animals due to exposure to antibiotics from various unknown sources. Antibiotics, even at low concentrations have been shown to exhibit ecotoxicological effects (Ngumba *et al.*, 2020; Pereira *et al.*, 2021). Increased detection is attributed to improper use and disposal of antibiotics, in addition to excretion of antibiotics through faeces and urine unchanged or as active ingredients of the antibiotics (Ngumba *et al.*, 2020). The antibiotics leach into groundwater from sanitation facilities like pit latrines and waste disposal facilities thus polluting the resource which is easily accessible thus prone to pollution. These informal settlements are densely populated and are under pressure to provide both quality sanitation facilities at the same time supplement the already scarce water sources for the population.

Antibiotics sulfamethoxazole, trimethoprim, and metronidazole, used for prophylaxis and treatment of infections, especially among people living with HIV/AIDS have been detected in one shallow well in Kisumu, Kenya. This is however not representative of the situation all informal settlements. Therefore, this study is important in that it gives a wider perspective of the problem in the study site. Mechanisms that trigger development of resistance and the spread of antibiotic resistance are also not well understood. Currently the prevalence of HIV/AIDS in the study area is 16.3%, which is above the national average of 4.9% in Kenya. It is in this light that it was imperative to study these antibiotics used to prevent opportunistic infections in the HIV/AIDS patients to be able to give direction on the risk they are likely to be exposed to if the environment, and especially groundwater was contaminated with antibiotics.

This study site sits on a shallow aquifer (water table) where groundwater is easily accessible, therefore used to supplement water shortages at household level oblivious of the possibility of the exposure to contaminants that may be in the water.

**CHAPTER TWO:****LITERATURE REVIEW****2.1 Occurrence of emerging organic contaminants in aquatic media**

A contaminant is any physical, chemical, biological, or radiologically active substance in water. The contaminants are not often monitored but end up in the environment and cause harm to aquatic life as well as humans (Cristina *et al.*, 2021; Geissena *et al.*, 2015). Some contaminants are expected to be at higher concentrations, posing risk to health, however, presence of contaminants does not necessarily mean a risk to health. Emerging organic contaminants (EOC's) are compounds, including original and by-products increasingly being detected in water as a result of developing analytical techniques (Lopez *et al.*, 2015; Noguera-Oviedo & Aga, 2016). Emerging organic contaminants include a range of hydrocarbons, personal care products as well as pharmaceutical products (Justino *et al.*, 2016; Richards *et al.*, 2021). The compounds end up in the environment and in water sources as a result of natural and anthropogenic activities (Akhtar *et al.*, 2021; Ujile, 2013). Some of the contaminants are transferred to organisms in the aquatic environment through feeds (Justino *et al.*, 2016). These compounds are known or are suspected to cause negative ecological effects and adverse effects to human health (Farré *et al.*, 2012; Serwecińska, 2020). Concentrations of emerging organic contaminants in the environment deserve more attention (Petrie *et al.*, 2014), to understand occurrence, fate and ecological impacts that include risks to human health. The release of pharmaceutical products into the environment raises concerns of harm to human health which manifest as anti-microbial resistance (Alderton *et al.*, 2021) or poisonous property to aquatic life, more-so since the contaminants are routinely monitored and in cases where they are monitored, removal during water treatment is not guaranteed (Geissena *et al.*, 2015; Rezka & Balcerzak, 2016), raising concerns of the ultimate effect on human health due to chronic exposure of the same compounds in the environment, it has been argued that exposure to low doses is of no effect to human health (WHO, 2011), but consideration need to be put in the chronic exposure of the compounds, over a period of time and among a vulnerable population, putting into consideration the effect of compounds as a result of transformation of the residues in water.

Until most recently, in the year 2015, the status of emerging organic contaminants remained unknown. Research on emerging organic contaminants is increasing, especially in the developing world, but little consideration, and almost no studies conducted in developing countries where populations struggle with inequalities as a result of shortage of essential services (K'oreje *et al.*,

2016; Petrie *et al.*, 2014). Furthermore, research on emerging organic contaminants is mostly conducted in surface water, with little known about groundwater (Meffe & de Bustamante, 2014). Emerging contaminants have also been detected in wastewater (Niemuth & Klaper, 2015). Their continued use and improper disposal end up as contaminants, that bound to be experienced in the environment (Sorensen *et al.*, 2015). The magnitude of contamination is coupled with population growth in the informal settlements caused by rapid urbanization (Oiro *et al.*, 2020; Pal *et al.*, 2014; Sorensen *et al.*, 2015). Lack of quality water has always been a determinant of health, and contribute to the current burden of communicable diseases, especially in the informal settlements. Inclusion of emerging contaminants in the priority list routinely monitored and removed during water treatment is important. This will ensure provision of good quality water, free of contamination by emerging organic contaminants (Lamastra *et al.*, 2016; Jacobsen *et al.*, 2005). However, to be able to routinely monitor the contaminants, they must be identified and quantified. Among the few studies conducted on emerging contaminants; and pharmaceutical products in groundwater, researchers point at possibilities of development of risk to human health and aquatic life (Lapworth *et al.*, 2012; Rodas-zuluaga *et al.*, 2020).

## **2.2. Occurrence of pharmaceutical products in groundwater**

Pharmaceutical products are categorized as an emerging group of pollutants, whose occurrence is not well known (Hughes *et al.*, 2013; Celle-Jeanton *et al.*, 2014). These pharmaceuticals are both human pharmaceuticals and for veterinary use (Jacobsen *et al.*, 2005). Contamination of surface water (rivers) as well as wastewater have been extensively studied, with a little known about contamination of groundwater sources, especially in Africa (Lapworth *et al.*, 2012). This is despite the knowledge that Africa suffers inequalities e.g. lack of basic resources like water and sanitation (Abayawardana & Hussain, 2002). Determination of the occurrence of studies on occurrence of pharmaceutical products in water in African water bodies has progressed, but still more has to be done to ensure protection of African water sources (Madikizela *et al.*, 2017). In most countries, groundwater is the only main source of water for domestic use and must be protected from contamination (K'oreje *et al.*, 2016; Sui *et al.*, 2015).

A wide range of pharmaceutical products have been detected in water all over the world in the recent past decade (Balakrishna *et al.*, 2017; Hughes *et al.*, 2013), with a partiality towards freshwater. Levels of concentration of pharmaceutical products vary among different water

sources (K'oreje *et al.*, 2016), and in some instances, the concentration of the pharmaceuticals detected exceed limits known to be lethal to aquatic organisms (Hughes *et al.*, 2013; Sui *et al.*, 2015) and also a potential threat to human health (Sui *et al.*, 2015; Mceachran *et al.*, 2017). This has resulted to attention shifting to determination of effects of chronic exposure to low concentration.

Different types of pharmaceutical products have been reported, both surface water (rivers), and groundwater sources. Studies on occurrence, sources and fate of pharmaceutical and personal care products in groundwater have found that the most frequently detected pharmaceutical products that included antibiotics (K'oreje, *et al.*, 2016; Nguyen *et al.*, 2015; Sui *et al.*, 2015; Wenjing *et al.*, 2016). This is in addition to other pharmaceuticals that include, anti-inflammatories, lipid regulators, carbamazepine, psychoactive stimulant (caffeine), antiretroviral medication, analgesics, psychiatrics medication, NSAIDs, beta-Blockers, Diuretics, PDE-V inhibitors, Iodinated contrast media (ICM), antipyretic drugs, anti-epileptic drugs; hormones, corticosteroids (Barnes *et al.*, 2008; Fick *et al.*, 2009; Vanessa de Jesus *et al.*, 2014; K'oreje *et al.*, 2016; Madikizela *et al.*, 2017; Matongo *et al.*, 2015; Mceachran *et al.*, 2017; Paíga & Delerue-Matos, 2016; Sui *et al.*, 2015).

Groundwater sources are vulnerable to contamination, which could either be through introduction of contaminants directly by surface run-off during rainy seasons (Sorensen *et al.*, 2015); or could be due to cross-contamination of the water sources by adjacent sanitation facilities e.g. pit latrines or sewer lines that may interact with the groundwater (Kimani-Murage & Ngindu, 2007). This could be termed as involuntary exposure since the pharmaceutical products end up in the environment without our knowledge (Daughton G, 2008); as opposed to exposure to pharmaceutical products disposed deliberately into the sewer system.

Waste water has been identified as the primary sources of pollutants discovered in groundwater sources. (Fick *et al.*, 2009; Koopaei & Abdollahi, 2017). In addition, hospital waste has also been found to be a source of pollution (Mendoza *et al.*, 2015), placing hospital waste management on the spot and necessitating an investigation into the safety of groundwater used in hospitals (if used). Drug manufacturing companies have also been found to add to the pollution of water sources through effluent release (Larsson, 2014a). The most unlikely source of contamination of groundwater sources have been cemeteries (Paíga & Delerue-Matos, 2016), with concentrations of up to 71 ng/L.

## 2.3 Occurrence of antibiotics

Antibiotics discovery and use since the 19<sup>th</sup> Century immensely improves human health, but in the long run the risk of antibiotic resistance have also developed (Hanna *et al.*, 2023; Roose-Amsaleg & Laverman, 2016; WHO, 2011b). Continued immense use in human beings and veterinary for growth promotion has led to occurrence of the same antibiotics in different environments in varied concentrations (Carvalho & Santos, 2016). Some antibiotics have known inherent toxic characteristics, but for others little is known. A wide range of antibiotics have been found in various aquatic media including wastewater, surface water as well as groundwater sources (Watkinson *et al.*, 2009), where specific antibiotics have been linked to interfere with biological processes e.g. nitrification, denitrification, and anammox (Roose-Amsaleg & Laverman, 2016), antibiotic resistance and antibiotic resistant genes (Casanova & Sobsey, 2016; Xu *et al.*, 2015). Antibiotics when ingested are mostly excreted without undergoing metabolism (Madikizela *et al.*, 2017) and their concentrations may be high, remaining in the environment like water for long periods of time. The antibiotics may end up in groundwater, and the long tenacity leading to continual and involuntary exposure to sub-optimal doses feared to favor and sustain antibiotic resistant genes (Allen *et al.*, 2017; Cizmas *et al.*, 2015; Rezka & Balcerzak, 2016; WHO, 2015; Zhang *et al.*, 2015).

### 2.3.1 Occurrence and concentration of Sulfamethoxazole, Trimethoprim and Metronidazole

Sulfamethoxazole, trimethoprim and metronidazole were most frequently detected antibiotics (Detection Frequency of 90%) among a group of pharmaceuticals targeted in water samples in Kisumu by K'oreje *et al.*, 2016, and at concentrations of between 5-50 µg/L<sup>-1</sup> in groundwater, in surface water, the concentrations were reported to be 39 µg/L<sup>-1</sup> for sulfamethoxazole and 7 µg/L<sup>-1</sup> for trimethoprim whereas in wastewater treatment plants the concentration of trimethoprim was reported to be 162 µg/L<sup>-1</sup>. Wastewater treatment plants as well as surface water have been found to be sources of groundwater contamination therefore, unprotected groundwater sources are mostly susceptible to contamination (Celle-Jeanton *et al.*, 2014; Chen *et al.*, 2014; Kosma *et al.*, 2014; Lien *et al.*, 2016; Matongo *et al.*, 2015). It is important to also note that sulfamethoxazole and trimethoprim are the most frequently detected antibiotics in water all the world (Beek & Weber, 2016).

In a review by Beek and Weber *et al.*, 2016, the two antibiotics have been detected in African aquatic environments: sulfamethoxazole at 2.53 µg/L<sup>-1</sup> and trimethoprim at 0.985 µg/L<sup>-1</sup>. The most

frequently occurring antibiotic in groundwater where forest land was applied with municipal wastewater in the USA was sulfamethoxazole at  $5 \mu\text{g/L}^{-1}$  but a higher concentration of sulfamethoxazole  $500 \mu\text{g/L}^{-1}$  and trimethoprim  $900 \mu\text{g/L}^{-1}$  were recorded in irrigation wastewater (Mceachran *et al.*, 2017). Of concern is that transformational compounds of the antibiotics (Kosma *et al.*, 2014), however, the antibiotics sulfamethoxazole and trimethoprim are stable in water due to their compact structure. The Limits of Detection (LOD), to achieve maximum detection of the contaminants is important to achieve objective of quantifying antibiotics in water as has been done for milk (Jiang *et al.*, 2013).

The fate of antibiotics when released into the environment, e.g. water is important. There are antibiotics that are highly volatile and soluble thus found in most environments (Daghrir, R., & Drogui, 2013) while others are not. Antibiotics were not detected in groundwater but were found to persist in surface water for up to 44 days in Mekong Delta in Vietnam, and the low levels were not found cause immediate harm to the environment (Nguyen *et al.*, 2015). The major concern however was the effect on aquatic life and human beings because of chronic exposure.

Health risk of exposure to individual antibiotics may not be possible, but a mixture effect may lead to elevated risks (Johnson *et al.*, 2015; Kumar *et al.*, 2010; Zhou *et al.*, 2016). There are however other antibiotics that cause harm to aquatic environments even when not in a mixture; e.g. triclosan is capable of causing up to 85% of bacterial death (Ricart *et al.*, 2010). Some mixtures of pharmaceuticals that include antibiotic trimethoprim can result to a Hazard Quotient that extends beyond 10 units (Mendoza *et al.*, 2015), indicating high risk of exposure. Sub-optimal levels of concentration of antibiotics is feared to not only lead to antibiotic resistance but also lead to other undesirable side effects on human beings (Rodríguez *et al.*, 2013).

Removal of antibiotics from water is not 100% possible meaning that there is continual involuntary exposure to contaminated water. For instance fluoroquinolone removal efficiency from wastewater was found to be 65% (He *et al.*, 2015; Janecko *et al.*, 2016). Removal efficiency for Metronidazole after wastewater treatment, has been found to be lower at 50% (Lien *et al.*, 2016).

## 2.4 Methods of detection

Contaminants of emerging threat have been detected in the environment, although arguments on the limit of detection to ascertain presence of the contaminants could arise. However, detection of

antimicrobials, including antibiotics has recently improved, with novel analytical techniques that allow for detection of antimicrobials even at low concentrations (Dasgupta & Sengupta, 2015). Methodologies used during the detection of antibiotics in different media (water and food samples) have been described by different researchers. In the detection of antibiotics in water, the most popular has been the SPE-LC-ESI-MS/MS method (Solid Phase Extraction coupled with Liquid Chromatography tandem Mass Spectrometry) (K'oreje *et al.*, 2016; Madikizela *et al.*, 2017; Ngumba, Kosunen, *et al.*, 2016; Ngumba, Gachanja, *et al.*, 2016; Okaru & Ndwigah, 2017). In food samples, advances in detection of antibiotics have been made, and immunoassays, microbiological tests and biosensors with a low limit of detection (LOD) (Cháfer-Pericás *et al.*, 2010). Another method is Rapid Resolution Liquid Chromatography coupled with a tandem mass spectrometer (RRLC/MSMS) after Solid-Phase Extraction (SPE) (Celle-jeanton *et al.*, 2014), whereby the Predicted Environmental Concentrations (PEC) and Measured Environmental Concentrations (MEC) used in the determination of hazards are compared to determine the most appropriate measure. Stability of the antibiotics in water is important, and preservation of samples for optimum detection and accurate determination of compounds (Llorca *et al.*, 2014).

## 2.5 Antibiotic resistance

Antibiotic resistance has been detected all over the world and is one of the greatest threats to global health today and it is increasing. It occurs when bacteria transform to change the way they respond to antibiotics (WHO, 2015). Resistance occurs naturally but is also propagated by misuse (WHO, 2015) and disposal that result to continued exposure to antibiotics either while undergoing treatment and currently through environmental exposure (Holmes *et al.*, 2016). Antibiotic resistance has always been viewed as a clinical problem that occurs in healthcare settings but other environments have been found to harbour antibiotic resistant bacteria with the resistant genes (Bjorn Berglund, 2015; Casanova & Sobsey, 2016; Hanna *et al.*, 2023). Occurrence of antibiotic resistance bacteria (ARB) is a serious public health problem globally as it threatens treatment of existing and emerging microbes (Serwecińska, 2020; Xi *et al.*, 2009). The development of this bacteria has been traced to the environment that include the soil and water, including groundwater (Casanova & Sobsey, 2016; Chen *et al.*, 2014).

Sulfamethoxazole and trimethoprim are often used in combination and most commonly detected antibiotic in most aquatic media and have also been found to have a high detection frequency and

(aus der Beek *et al.*, 2016; K'oreje *et al.*, 2016). Resistance to sulfamethoxazole and trimethoprim (a combination known as cotrimoxazole) has been reported in clinical settings towards HIV positive paediatric patient in Lima, Peru (Romero *et al.*, 2010). Selective resistance for trimethoprim in aquatic environments (Kraupner *et al.*, 2020) has been reported but not for sulfamethoxazole.

Metronidazole has been used for long in the treatment of various infections in human being caused by anaerobic and microaerophilic bacteria (bacteroides, clostridia, helicobacter) as well as parasites (trichomonas giardia, entamoeba) whereby resistance have been reported (Dhand & Snyderman, 2009). There are however, gram-positive bacteria that are known to be naturally resistant to metronidazole e.g., lactobacilli. Sensitivity test for metronidazole is not commonplace, leading to underreporting of resistance to metronidazole. There are however reports of determination of resistance to metronidazole in-vitro among different organisms that include *Bacteroides fragilis*, *Helicobacter pylori*, *Trichomonas*, *Clostridium difficile*, *Entamoeba* and *giardia* (Dhand & Snyderman, 2009).

### **2.5.1 Effect of exposure to antibiotics**

Prevalence of HIV/AIDS in the study area has been reported to be high; 3.4 times that reported national prevalence of 19.9% (MoH, 2016). It is the same population that suffer opportunistic infections due to their compromised immunity. There are set guidelines (Centers for Disease Control and Prevention (CDC), 2017) on the CD4 cell count level at which patients are introduced to antibiotics for prophylaxis and failure to adhere to the guideline, this patients' chances of dying are 10% if antibiotics are not administered to tuberculosis (TB) patients (Hakim *et al.*, 2017). Groundwater in these areas have been found to contain appreciable levels of antibiotics used for prophylaxis, in addition to ARVs (K'oreje *et al.*, 2016; Ngumba *et al.*, 2020). This raises questions of if there are health effects as a result of exposure to low doses of the same drug that is supposedly required to protect from infections, and if there are effects, they should be quantified.

### **2.5.2 Methods of determining antibiotic resistance**

Antibiotic resistance is determined by assessing bacteria sensitivity to antibiotics through microbiological assays, which are techniques that involve diffusion of antibiotics or dilution of antibiotics in agar or in broth (Dafale *et al.*, 2016). There are different designs of microbiological assays which include but not limited to the following: Kirby- Bauer disk diffusion method, broth dilution method, agar dilution method, E-test, and agar diffusion method.

#### **a. Kirby-Bauer diffusion method**

This is commonly used to determine bacteria sensitivity to antibiotics. Bacteria are grown on Mueller-Hinton agar on filter paper disks impregnated with antibiotic of interest (Reddy, Chavi *et al.*, 2017). The drug diffuses through the agar, with the drug concentration decreasing with increase in the distance from the disk (Sykes & Rankin, 2014). No bacteria growth around the disks indicates ability of the antibiotics to prevent bacteria growth, therefore not resistant. Interpretation of the zone diameters is based on standards and the organisms are termed susceptible, intermediate or resistant (Sykes & Rankin, 2014). Used for rapidly growing bacteria.

#### **b. Broth dilution method**

This method comprises of bacteria isolates subjected to a series of different concentrations of antibiotic in a broth. There are two types of test in this method one consisting of microdilution testing uses about 0.05-0.1 ml of total broth and can be performed in a microtitre plate (Reddy *et al.*, 2017). The other type of test is macrodilution testing which uses up to 10ml of broth and is performed in a standard test tube (Reddy *et al.*, 2017). Minimal Inhibitory Concentration (MIC) is reported as the lowest concentration at which bacteria is completely inhibited.

#### **c. Agar dilution method and Agar diffusion method**

Agar dilution the antibiotic is incorporated into a solid agar medium in a petri dish. A standard concentration of the bacteria organisms inoculated on the surface of the medium. Susceptibility of the bacteria to the antibiotic is indicated by no growth (Reddy *et al.*, 2017). It provides a specific MIC of an antibiotic.

On the other hand, agar diffusion uses a cylindrical-plate or Cup in determination of antibiotic potency and bioactivity. It depends on the diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish. Inhibition of growth of microorganism appears on a circular area around the cylinder containing the antibiotic.

#### **d. E-test (Epsilometer test)**

A quantitative method of testing the susceptibility of an antimicrobial agent. A thin plastic carrier strip coated with an antimicrobial gradient is applied to one side and a minimum inhibition concentration (MIC) interpretative scale on another side. An agar plate is inoculated with the bacteria of interest to enable growth of a layer of the bacteria (Sykes & Rankin, 2014). The strips are smeared to the surface of the plate. This is then incubated, and let to produce an antimicrobial ellipse, which is the zone of inhibition. The MIC value is indicated by an intersection of the

inhibitory zone comparing with the MIC scale on the carrier strip, or the standard. This method is precise and accurate and useful in fastidious and anaerobic bacteria (Sykes & Rankin, 2014).

## **2.6 Routes of groundwater contamination with antibiotics**

Antibiotics have been found to enter the environments through various points termed as points of accumulation. This points the groundwater contamination with antibiotics have been identified to be hospital waste disposal, wastewater used for irrigation, sewage, landfills, animal waste and pit latrines (Azanu *et al.*, 2018; Daughton G, 2008; Lamastra *et al.*, 2016; Mceachran *et al.*, 2017; Pan & Chu, 2018).

The contribution by human beings in contamination of groundwater sources with antibiotics have not been determined. There are however studies linking pit latrine to groundwater contamination with antibiotics (Abdulkadir *et al.*, 2015; Ahaneku & Adeoye, 2014; Graham & Polizzotto, 2013) whereby the antibiotics are excreted degraded or unchanged ending up in groundwater. To the best of our knowledge human direct disposal of antibiotics at household level has not been explored as a possible source of antibiotic contamination in groundwater and a risk factor to development of antibiotic resistance.

Community contribution to contamination of groundwater sources with antibiotics and level of contamination through direct disposal has not been determined. Community understanding of the vulnerability of the aquifer need to be determined at household level to develop means of leveraging the community's ability to prevent pollution of the water sources for their wellbeing.

## **2.7 Human health risk of exposure to antibiotics in the environment**

Presence of antibiotics in aquatic media and groundwater have been studied, but the effect of low level chronic exposure is barely known (Janecko *et al.*, 2016); far less is known on effect on human beings. Human health risk associated with exposure to antibiotics in the environment have been characterized in aquatic environments for other contaminants with little known about exposure to pharmaceutical compounds in general (Koopaei *et al.*, 2017). Sources of contamination harbor contaminant whose teratogenicity, toxicity and carcinogenicity are unknown and therefore of public health importance as they are a health hazard to human. Wastewater has been found to be a main source of contaminants, a disadvantage in that it cannot be used as a supplementary source of water for deserving populations especially in LMICs. The inapplicability is as a result the

prohibiting allied costs of treatment as well as lack of technologies to treat the water (Chen *et al.*, 2016).

The situation is not different when wastewater is used for irrigation as the contaminants get to the human being through the plants and fish, therefore contamination is inevitable (Nguyen *et al.*, 2015; Chen *et al.*, 2014). With antibiotics as a pollutant, the biggest fear is that of development of antibiotic resistance, often associated with exposure to antibiotics (Cizmas *et al.*, 2015; Fick *et al.*, 2009; Marti *et al.*, 2014; Martinez, 2009; Rezka & Balcerzak, 2016; WHO, 2015).

Risk Assessment is conducted as a result of exposure to harmful substances, where risk is a function of hazard (how toxic the substance is) and exposure (how much has been taken). Human exposure to environmental contaminants has been determined using Risk Quotients (RQ) but no appreciable risk of exposure to antibiotics was found (Vanessa de Jesus *et al.*, 2014). However, the risk of exposure is dependent on the antibiotics, the levels of concentration and to a great extent the sources (Koopaei & Abdollahi, 2017). Environmental risk assessment of exposure to antibiotics among aquatic life has been based on consumption of antibiotics, with risk being found to be low (Iatrou *et al.*, 2014) but recommends use of toxicity mixtures as opposed to using baseline toxicity measurements (Ecological Structure Activity Relationship) ECOSAR (González-Pleiter *et al.*, 2013).

Risk assessment on vulnerable populations has not been determined as far as exposure to antibiotics via drinking water is concerned. The various groups of vulnerable populations e.g., children under the age of 5 years, pregnant women, geriatrics, and HIV/AIDS patients. The proposed study site (Kisumu, Kenya) bears a burden of negative health outcomes among them being a high prevalence of HIV/AIDS, which is 3.3 times higher than the national prevalence of 16.3% (NACC, 2018). This population depends on provision of medication including (antiretroviral medication) ARVs and antibiotics from the government to reduce the burden of disease and opportunistic infections that lower immunity. The same medications provided have been found in water including groundwater (K'oreje *et al.*, 2016) in concentrations that warrant attention by determining the risk among this populations.

### **2.7.1 Human health risk assessment (HHRA)**

Risk posed by chemical compounds is determined considering the toxicity, population exposed, the quantities and the route of exposure. When conducting a human health risk assessment, a toolkit that describes the likelihood of exposure and how the risk assessors manage the risk is used.

The toolkit presents a set of questions an assessor follows to gather information on exposure. The risk assessment process consist of steps as follows (WHO, 2021; WHO, 2009).

**a. Hazard identification**

Here, the identity of the chemical and potential hazardous properties of the chemical is determined.

**b. Hazard characterization and guidance/ guideline value identification**

In this step the properties of the chemical with potential to cause adverse health effects are determined. The existence of guideline values from international organizations is determined. The assumptions about exposure and dose stated in the international guidelines are checked and whether the assumptions reflect conditions specific population being assessed.

**c. Exposure assessment**

This step is concerned with the ways the population come into contact with chemical compounds, the magnitude of exposure, the exposure period and the measure of exposure to necessitate health risk characterization.

**d. Risk characterization**

In this step the estimated exposure is compared with the standards/ guideline values given by international organization for the chemical. *Table 1* summarizes the steps in risk assessment and expected output.

**Table 1: Questions asked in the process of risk assessment and expected outcomes**

| Question                                                                                                                                                                                                                                                                                                                                                                             | Expected output                                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Hazard identification</b>                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                        |
| What is the identity of the chemical?<br>Is the chemical potentially hazardous to humans?                                                                                                                                                                                                                                                                                            | A clear identification of the chemical (antibiotics)<br>Depiction of health hazards from internationally available information and publications                                                                                                                                                                                                                                                        |
| <b>Hazard characterization</b>                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                        |
| What is the chemical composition that has the potential to cause adverse health effects?<br>Is there guidance or guideline values from international organizations for the chemical?<br>What assumptions on exposure and dose are described by the available guidance/ guideline values for the chemical?<br>Do the assumptions reflect conditions specific to the local population? | Qualitative or quantitative description of the properties of the chemical with potential to cause adverse health effects<br>Guidance values (rates or concentrations) for the chemical obtained from internationally available resources<br>List of assumptions about the contact rates and absorption<br>A reference value reflecting exposure and dose parameters specific to the given demographics |

|                                                                                                                                                          |                                                                                                                                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Exposure assessment</b>                                                                                                                               |                                                                                                                                                                                      |
| What are the possible ways of people coming into contact with the chemical?<br>What measure of the exposure is required for characterizing health risks? | Qualitative description of relevant media/ routes of exposure<br>Determination from given guidance values of whether an exposure rate is needed to perform the risk characterization |
| <b>Risk characterization</b>                                                                                                                             |                                                                                                                                                                                      |
| What is the comparison of the estimated exposure with guidance/ guideline values for the chemical?                                                       | A quantitative or qualitative statement of non-risk (e.g. Hazard Quotient)                                                                                                           |

Source: (WHO, 2009).

Hazard Quotient (Mendoza *et al.*, 2015), is a measure of assessing risk computed for non-carcinogenic compounds. These are substances are tolerated since the body is able to recover from exposure; and antibiotics can be considered part of this substances. For non-carcinogenic substances, risk is determined as a Hazard Quotient (HQ) as follows:

$$HQ = \frac{I_{non - carcinogenic}}{RfD}$$

Where, I= Intake Rate

$$I = C \frac{CR \times EF \times ED}{BW \times AT}$$

C= average concentration of contaminant at exposure (mg/L in water)

CR=contact rate (L/day)

EF= exposure frequency (days per year)

ED= exposure duration (in years)

BW= body weight (Kg)

RfD= Reference Dose Factor (a ratio of the No-Observable Adverse Effect Level (NOAEL) divided by the Uncertainty Factor (UF)). This are values provided in tables.

HQ< 1; means that the water is safe

HQ>1; means that the water is unsafe

A sum of the individual HQs is taken in a case where several substances are detected in the water.

**Conclusion**

From the literature review, the presence of antibiotics in the environment have been linked to development of antibiotic resistance as well as development of resistant genes that are passed through many generations of bacteria. The antibiotic contaminants get to groundwater through the environment from varied sources that include hospital effluent, interaction of groundwater with blocked or faulty sewer lines, proximity to pit latrines and direct disposal of uncompleted antibiotic doses. Vulnerability of groundwater sources to contamination is facilitated by accessibility to high water table (aquifer), which is the case with this study site. Complicating the situation is the current water treatment techniques that do not include removal of antibiotic residues, exposing consumers of the water to a possibility of exposure to API's and their metabolites. This necessitates inclusion of pharmaceutical products in the routinely monitored compounds during water treatment.

## **CHAPTER THREE:**

## **MATERIALS AND METHODS**

### **3.1 Study design**

This was a descriptive cross-sectional study, of households in informal settlements of Kisumu, Kenya using groundwater for domestic purposes. Groundwater sources were located and mapped using a geographic information system (GIS). GIS is a computer system generated to capture, store, manipulate, analyse, manage, and present all types of geographical data to locate the exact location of groundwater sources and eventually develop a visual picture (map) of the groundwater points (boreholes, shallow wells, and springs) within the study site in relation to potential sources of contamination. The number of water points for sampling were determined and selected from each of the settlements.

### **3.2 Study area**

The study was conducted in Kisumu County located in the Western side of Kenya, 350km from Nairobi within longitudes 33° 20'E and latitude 0° 20'S and 0° 50'S (Kisumu, 2018). Kisumu sits on 2,086km<sup>2</sup> total land area and approximately 567km<sup>2</sup> is water. The County is situated on the shores of Lake Victoria which is the third largest freshwater lake in the world shared by Kenya, Uganda, and Tanzania. The study sites are in Kisumu East and Kisumu Central sub-counties. There are 8 informal settlements in the County, out which 5 were studied and they included, Obunga, Manyatta A, Manyatta B, Nyalenda A and Nyalenda B.

The informal settlements are estimated to have a population density of approximately 567,983 (Statistics, 2019), with an annual urbanization rate of 2.8 percent (Kisumu Sustainable Mobility Plan, 2021). The population in the informal settlements rely on groundwater to supplement their need for water for domestic purposes and at the same time use the sub-surface for sanitation facilities where the majority use pit latrines (Kanoti, Olago, Opiyo, & Nyamai, 2019).

We mapped the groundwater points in the study area, where water samples were collected from 49 groundwater sources distributed across the five informal settlements namely, (Obunga, Manyatta and Nyalenda). *Figure 6* shows the map of informal settlements and groundwater sources where water samples were collected.

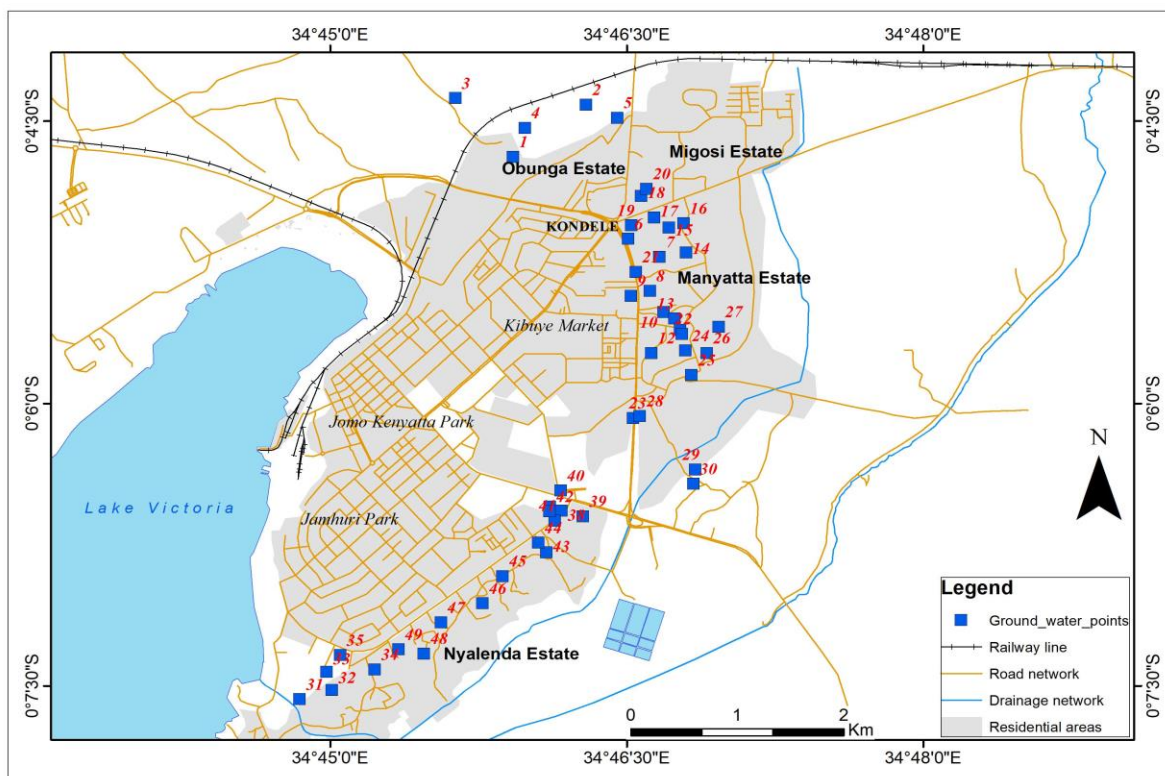


**Figure 6: Map of study area showing water points around which the household were selected.**

There were an estimated 38,964 households in the informal settlements selected for study (Population Census, 2009). The Kenya National Population and Household Census of 2009 estimated the population to be 150,124.

The distribution of household in informal settlements is as shown in *table 2*.

**Table 2: Population per informal settlement.**

| Name of Informal Settlement | Number of Households |
|-----------------------------|----------------------|
| Manyatta A                  | 12,525               |
| Manyatta B                  | 7,808                |
| Nyalenda A                  | 8,070                |
| Nyalenda B                  | 8,561                |
| Obunga                      | 2000*                |
| <b>Total</b>                | <b>38,964</b>        |

Source: Population Census 2009

\*approximate

### 3.3 Target population and study population

The target population was informal settlements of Kisumu, Kenya that used groundwater for domestic purposes. The first study population was households within the study area. A structured questionnaire was administered to head of households to assess patterns of antibiotic use, disposal and knowledge of health effects of exposure to antibiotics through consuming water contaminated with antibiotics. The second study population was groundwater points. Water samples were collected from previously mapped groundwater sources for analysis to determine the antibiotics concentration and presence of antibiotic resistance. Microbes were isolated and the presence of antibiotic resistant microorganisms (*E. coli*) in the groundwater samples was determined using culture-dependent methods (Xi *et al.*, 2009) against antibiotics sulfamethoxazole and trimethoprim.

A Human health risk of exposure was conducted to establish level of risk of exposure to antibiotics at household level using the World Health Organization Human Health Risk Assessment toolkit for chemical hazards (Ashbolt *et al.*, 2013; World Health Organization, 2010). This particular toolkit was modified to suit the particular contaminants and adopted for use in antibiotic contamination in groundwater in Kenya.

Key informants selected from Kisumu County health officials and County officials responsible for water quality in the County were interviewed to give information on the protection given to residents regarding contamination of groundwater sources with antibiotics.

### 3.4 Inclusion/exclusion criteria

#### Inclusion criteria

- All groundwater sources were included in the study for purposes of mapping as long as the owners gave consent to the exercise.

The owners of these groundwater sources were those who bought pieces of land in the study area and built residential houses, which were mostly rental. These owners were initially contacted during the mapping of the groundwater points and were informed that a questionnaire would be administered, and water samples collected on a later date. All heads of households who used groundwater and gave informed consent were included in the study.

#### Exclusion criteria

- All households that did not consume groundwater.

- All groundwater sources that were not documented during the initial mapping of the groundwater sources.

### 3.5 Sampling and sample size determination

#### 3.5.1 Sampling of water sources

Groundwater sources were mapped between 13<sup>th</sup> and 18<sup>th</sup> May 2018. The total number of groundwater sources mapped were 188 and distributed in all the five informal settlements selected for study. The groundwater sources were grouped according to the type i.e., boreholes, wells and springs as shown in *table 3*. The groundwater sources were proportionately selected for inclusion into the study depending on the number in the informal settlements as shown in *table 3*.

**Table 3: Number of groundwater sources per informal settlement**

| Informal settlement | Type of groundwater source | Number | Total |
|---------------------|----------------------------|--------|-------|
| Obunga              | Springs                    | 2      | 19    |
|                     | Shallow wells              | 17     |       |
|                     | Borehole                   | 0      |       |
| Manyatta A          | Springs                    | 1      | 67    |
|                     | Shallow wells              | 66     |       |
|                     | Borehole                   | 0      |       |
| Manyatta B          | Springs                    | 0      | 34    |
|                     | Shallow wells              | 33     |       |
|                     | Boreholes                  | 1      |       |
| Nyalenda A          | Springs                    | 13     | 43    |
|                     | Shallow wells              | 30     |       |
|                     | Boreholes                  | 0      |       |
| Nyalenda B          | Springs                    | 6      | 26    |
|                     | Shallow wells              | 20     |       |
|                     | Boreholes                  | 0      |       |

There were total of 188 mapped groundwater sources in the 5 informal settlements. From each informal settlement, groundwater sources were selected systematically to ensure proportionate

representation depending on the total number of groundwater sources in each settlement. The 188 groundwater sources were treated as a sampling frame and selection done randomly by selecting every 5<sup>th</sup> water point to ensure representation of all the informal settlements. This way informal settlements with more water point ended up with more water points included in the study. The selection of the water samples generated water points from all the informal settlements as follows; Obunga- 5, Manyatta (Manyatta A- 17, Manyatta B)- 8, and Nyalenda (Nyalenda A- 11, Nyalenda B- 8). A total of 49 groundwater samples were studied.

### 3.5.2 Household sample size determination

Households were selected based on groundwater use and included in the study. The number of households using the groundwater was calculated using the formula for proportions (Pourhoseingholi, Vahedi, & Rahimzadeh, 2013), and sample size determined as follows:

|                                 |                                                                                                                                                                                                                                                                                               |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| $n = \frac{Z^2 [P (1-P)]}{d^2}$ | <p>n= the required sample size</p> <p>Z= critical value associated with significance level of 95%, is 1.96</p> <p>P= the estimated proportion of households is estimated at 50% as no previous studies have been carried out</p> <p>d= the margin of error accepted for this study was 5%</p> |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|                                           |                                                                                                                                        |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| $n = \frac{1.96^2 [0.5 (1-0.5)]}{0.05^2}$ | <p>=384.16</p> <p>n=384.16+ {384.16*(15/100)}</p> <p>=384.16+57.624</p> <p>=441.784</p> <p style="text-align: center;"><b>=442</b></p> |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|

Based on the computed sample size 384, a 15% non-response rate was factored to give the required sample size of 442 heads of households.

From each informal settlement, households were then selected based on the population of each informal settlement to achieve the calculated sample size. Number of households were selected as shown in *table 4*.

**Table 4: Proportionate selection of households**

| <b>Informal settlement</b> | <b>Estimated Sample size</b> |
|----------------------------|------------------------------|
| Manyatta A                 | 136                          |
| Manyatta B                 | 84                           |
| Nyalenda A                 | 87                           |
| Nyalenda B                 | 93                           |
| Obunga                     | 22                           |

Households were randomly selected around the mapped groundwater points and the samples were proportionately selected among the five informal settlements as shown in *table 4*.

Households in the informal settlements are not numbered, therefore, households included in the study were selected systematically by identifying a starting point in the settlements. The starting point was an administrative unit i.e., the chief's office which were found in all settlements. From the starting point, every 5<sup>th</sup> household was systematically selected until sample size was achieved. Key informants selected were purposively selected among the County officials responsible for water and sanitation as well as health officials on their knowledge about contamination of groundwater sources with antibiotics. Measures taken to prevent contamination of water with antibiotics were also sought from the officials.

### **3.6 Data collection**

Prior mapping of the groundwater sources was done, and households around these water sources selected. The heads of the households at the time of data collection were interviewed to assess practices around antibiotic use and disposal and knowledge of health effects of consuming water that is contaminated with antibiotics. Water samples were collected from groundwater sources selected based on the 188 groundwater sources. A Human Health Risk Assessment was conducted among household members around groundwater points within the informal settlements selected for study by observation using a World Health Organization Risk assessment tool and Hazard Quotients (HQs) were determined. Interviews were also conducted among key informants in the County leadership concerned with health, environment, water, and natural resources.

All quantitative data collected was analysed using SPSS version 20 and STATA 14. Qualitative data was sorted and summarized according to emerging themes on the subject were reported.

### 3.6.1 Household interviews

Interviews were conducted among a random sample of households. The head of the house present at the time the interviewer visited the household was requested to consent to the study and information on antibiotic use and disposal among households was collected using a structured interviewer questionnaire. The interviews were conducted by the principal investigator with help from trained research assistants.

### 3.6.2 Groundwater collection

Groundwater collection depended on the techniques of drawing water by the World Health Organization (WHO, 2011a).

- a. Collecting water samples from a hand/ electric borehole or well fitted with a pump. For boreholes or wells that had a pump, the pump was operated continuously for at least 5 minutes to ensure that the water in the pipe was flushed. While taking care not to introduce contamination into the sterile water collection bottle, water was allowed to flow directly into the sterile water collection bottle (100ml bottle). The cap of the water collecting bottle was replaced immediately to avoid contamination of the water.
- b. Collecting water samples from an open well, where the water was only collected using a bucket, a sterile bottle attached to a weight was used. A strong rope or string was attached to the bottle and a weight attached just below the bottle. The cap on the sterile bottle was then opened and weight and the bottle lowered into the water to a depth of about one meter. Bubbles from the bottle as a result of water getting in were observed and the bottle was withdrawn, and the lid replaced to avoid contamination.
- c. Collecting water samples from a spring, where water flows. After opening the lid of the sterile water collection bottles, water was allowed to flow into the bottle until full, holding the base of the water bottle. The lid was replaced immediately to prevent contamination.

### 3.6.3 Sample handling

- Sticker labels with unique codes were prepared along with a data capture form. Upon collection of the samples, the stickers were placed on the water bottles to avoid sample mix-up. Water samples were collected in triplicate for the purposes of preparing standards before analysis, determination of concentration of antibiotics, isolation of *E. coli* and *C. Parvum* and determination of antibiotic resistance in *E. coli* isolates.

- The samples were placed in insulated cool box maintained at 4 °C for preservation before transportation immediately to the Kenya Bureau of Standards laboratory in Kisumu. The water samples were transferred into a refrigerator and stored at a temperature of -20 °C prior to transportation to the Kenya Bureau of Standards laboratory in Nairobi. Water samples for determination of antibiotic concentrations are required to be analyzed immediately after collection but in our situation, where the analysis was not possible immediately, the samples remained stable under preservation at -20 °C (Llorca *et al.*, 2014). The reagents for analysis were procured by the Kenya Bureau of Standards (KEBS).

### 3.7 Water sample analysis for determination of concentrations of antibiotics

#### Preparation of Reagents and Chemicals

##### *Chemicals and standards*

Analysis for antibiotic concentrations (sulfamethoxazole, trimethoprim and metronidazole) was done using a Liquid Chromatography (LC-MS/MS-ESI), a method that combines the resolution of high-performance liquid chromatography (HPLC) separation with the high mass accuracy of the mass spectrometer. In preparation for analysis, the samples were filtered through glass fibre filters (1 µm) and PTFE/nylon filters (0.2 µm) from Agilent Technologies.

All antibiotic standards were of high purity grade (>90%) purchased from Sigma Aldrich. The mixed standard stock solution was prepared in methanol/water (50:50, v/v) to a concentration of 1000 mg/l for each of the three analytes. After preparation, the stock solution was stored at -80°C (KEBS Protocol). The spiked samples for recovery yield analysis were prepared by adding 10 µL, 125 µL and 250 µL of the 1 ppm stock solution into 250 ml of water resulting in final concentrations of 10 µg/kg (ppb), 50 µg/kg (ppb), and 100 µg/kg (ppb).

Agilent Bond elute Plexa cartridges (60 mg, 3 ml; Part number: 12109603) from Agilent Technologies were used for solid phase extraction.

LCMS grade methanol, acetonitrile, water, and formic acid 98%, were purchased from Merck (Darmstadt, Germany). EDTA and Hydrochloric acid 37% were purchased from Sigma-Aldrich and nitrogen for drying was generated in the laboratory.

***Sample processing (Sample cleanup and Pre-concentration)***

The sample cleanup and pre-concentration for the aqueous solutions was carried out following the protocol by (Rodriguez-Mozaz *et al.*, 2020) modified to be conducted via automated SPE.

The water was filtered by glass fibre filters (1 µm) followed by 0.45 µm PTFE/nylon filter. An appropriate volume of 0.1M Na<sub>2</sub>EDTA solution was added to the filtered water to get to a final concentration of 1g/L (g solute/ g solution) and the pH adjusted to 2.5 with hydrochloric acid 0.1 M. The samples were further processed by filtering through a Whatman filter paper to a 250 ml conical flask.

Solid phase extraction (SPE) was performed according to Rodriguez-Mozaz *et al.*, (2020). Briefly, Agilent Bondelut Plexa cartridges (60 mg, 3 ml; Part number: 12109603) cartridges were first conditioned with 6 mL methanol followed by 6 ml deionized water (UPLC grade, and pH adjusted to 2.5 with HCl 0.1 M). 100 ml of the sample was passed through the SPE cartridge samples at approximately 1 mL/min. Each cartridge was cleaned with 6 ml HPLC water (under gravity conditions) and dried under vacuum conditions for 10–15 min. For analyses, the samples were eluted from the cartridge with 6 mL of ultra-pure methanol, evaporated under N<sub>2</sub> stream near dryness, and reconstituted with 1 mL of mixture of methanol and water (50:50, v/v). Recovery values of the extraction method were calculated on each occasion (each sampling campaign) and used to correct the quantification values obtained using these calibration curves. To determine the recoveries, wastewater effluent was spiked in triplicate with a standard mixture containing all antibiotics at a final concentration of 10 ng/L. Recoveries were determined by comparing the initial concentrations after spiking with the concentrations obtained after the whole SPE procedure. Method detection limits (MDL) and method quantification limits (MQL) were also determined in each sampling campaign and for each antibiotic as the minimum detectable amount of compound with a signal-to-noise of 3 and 10, respectively.

The instrument conditions and multiple reaction monitoring parameters were as shown in *table 5 and 6*.

**Table 5: Instrument conditions of the solid-phase extraction and liquid chromatography (SPE-LC-MS/MS) used in the analysis of the groundwater samples.**

| Instruments                          | Conditions                                                                         |    |    |
|--------------------------------------|------------------------------------------------------------------------------------|----|----|
| Agilent 1290 Infinity II UPLC system | 1290 Infinity II binary pump with degasser                                         |    |    |
|                                      | 1290 infinity II autosampler with needle seat backflush function                   |    |    |
|                                      | Column temperature compartment                                                     |    |    |
|                                      | Agilent Zorbax Eclipse plus RRHD C18 column (2.1mm x 150 mm, 1.8µm) p/n 959759-902 |    |    |
|                                      | Reverse Phase Column                                                               |    |    |
| Mobile Phases                        | a) Water + 0.1% Formic acid                                                        |    |    |
|                                      | b) Methanol                                                                        |    |    |
| Flow rate                            | 0.30 ml/min                                                                        |    |    |
| Column Temperature                   | 35°C                                                                               |    |    |
| Injection volume                     | 20 µL                                                                              |    |    |
| Post time                            | 3 minutes                                                                          |    |    |
| Gradient                             | Time (min)                                                                         | %A | %B |
|                                      | 0                                                                                  | 90 | 10 |
|                                      | 8                                                                                  | 60 | 40 |
|                                      | 12                                                                                 | 35 | 65 |
|                                      | 13                                                                                 | 5  | 95 |
|                                      | 16                                                                                 | 5  | 95 |
| MS                                   | Agilent 1290 Infinity II UPLC with Agilent 6460C TQQQ (Triple Quadrupole Detector) |    |    |
| Ion source                           | Agilent Jet Stream (AJS)                                                           |    |    |
| Ionization mode                      | Positive                                                                           |    |    |
| Capillary voltage                    | 3500 V                                                                             |    |    |
| Nozzle Voltage                       | 500 V                                                                              |    |    |
| Nebulizer Gas (N2) Pressure          | 30 psi                                                                             |    |    |
| Drying Gas (N2) Temperature          | 325 °C                                                                             |    |    |
| Drying Gas Flow Rate                 | 6 L/min                                                                            |    |    |
| Sheath Gas (N2) Temperature          | 350 °C                                                                             |    |    |
| Sheath Gas Flow Rate                 | 11 L/min                                                                           |    |    |
| Acquisition Mode                     | MRM                                                                                |    |    |

**Table 6. The multiple reaction monitoring parameters**

| Target Compound | RT <sup>a</sup> | Precursor Ion [M + H] <sup>+</sup><br>(m/z) (CV) <sup>b</sup> | Quantifier Ion<br>(m/z) (CE) <sup>c</sup> | Qualifier Ion |
|-----------------|-----------------|---------------------------------------------------------------|-------------------------------------------|---------------|
|                 |                 |                                                               |                                           | (CE)          |
| SMX             | 4.74            | 254 (110)                                                     | 156 (12)                                  | 108 (24)      |
| TMP             | 4.24            | 291.1 (60)                                                    | 123.1 (30)                                | 80.9 (25)     |
| MTZ             | 3.32            | 172 (90)                                                      | 127.9 (10)                                | 82.1 (25)     |

. SMX = Sulfamethoxazole, TMP = Trimethoprim, MTZ = Metronidazole

<sup>a</sup>RT - retention time. <sup>b</sup>CV collision voltage. <sup>c</sup>CE collision energy

### 3.8 Isolation of *Escherichia coli* and determination of antibiotic sensitivity

*Escherichia coli* was detected using an enzyme substrate *E. coli* Test which uses proprietary Defined Substrate Technology (DST) to detect coliforms and *E. coli* simultaneously. The test consists of two nutrients indicators which are the major sources of carbon colilert and can be metabolized by the *E. coli* form  $\beta$ -galactosidase and *E. coli* enzyme  $\beta$ -glucuronidase respectively. Test reagent was added to 100ml of water sample in a sterile glass bottle and allowed to stand for 10 minutes (until the reagent dissolved). The mixture was then poured into a tray and the contents sealed with a Quanti tray sealer. This was placed in an incubator for 24 hours with at a temperature of 35° C  $\pm$  0.5°. The wells were counted, and positive samples identified by their fluorescent colour. The Most Probable Number table was used to estimate the *E. coli* counts.

The confirmed isolates were then subjected to further culturing to achieve well isolated colonies in nutrient agar plates. These well isolated colonies were further diluted in normal saline. The size of the inoculated organisms was standardized using the McFarland's 0.5 solution. Colonies were grown and picked from the samples and moved to a 3 ml of sterile distilled water to prepare bacterial suspension. Portions of 100  $\mu$ L of the suspension was used to spread on the Muller Hinton agar and incubated for 24 hours at 37 °C (Mulamattathil, Bezuidenhout, Mbewe, & Ateba, 2014). A pure culture of *Escherichia coli* bacteria was tested for antibiotic susceptibility against Sulfamethoxazole and Trimethoprim using Kirby-Bauber disk diffusion method (Mulamattathil, et al., 2014, Bauer A W, 1966). Antibiotic Inhibition Zone Diameters (IZD) were observed and recorded to determine antibiotic resistance. Susceptibility of bacteria was determined by ability of bacteria to develop around the thin substance containing antibiotics. The sensitivity status of the bacteria is determined by measuring the Inhibition Zone Diameter to the nearest whole millimetre,

which defines the bacteria as resistant ( $\leq 9$  mm), moderately sensitive (10-11 mm) or sensitive ( $\geq 12$  mm) (Addo-mensah & Holland, 2022). The area where bacteria did not grow were clear and referred to as a zone of inhibition and the larger the zone of inhibition, the more susceptible the bacteria and the smaller the zone the less susceptible the bacteria, signifying resistance.

### **3.9 Isolation of *Cryptosporidium*, detection, and determination of antibiotic sensitivity**

Total High Molecular weight DNA was extracted using QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Exactly 60 ml of water samples were pumped through a Filtia-Max™, a pre-treatment was done by transferring filter membranes into 15ml falcon tube and dissolved in 1400  $\mu$ L of ASL buffer from the DNA extraction kit. The samples were then exposed to five cycles of freeze and thaw in liquid nitrogen and boiling water to disrupt the oocysts. Afterwards, 700  $\mu$ L of ASL buffer was added into the sample tube and then the procedure was followed as per the Qiagen kit. Total genomic DNA was visualized on 1% agarose gel (Qiagen, 2010).

#### **Primer designing and Nested PCR amplification**

A section of 18S ribosomal RNA gene from *Cryptosporidium* genus under NCBI, Accession Nr. GQ259149.1 was selected and used to design specific primers for Universal qualitative detection of all *Cryptosporidium* species. Target DNA amplicons with 5' GGTGACTCATAATAACTTTACGG 3' as forward and 5' ACGCTATTGGAGCTGGAATTAC 3' as reverse primers. To reduce non-specific binding a Nested PCR was performed by the following primers using 5' TAAACGGTAGGGTATTGGCCT 3' as forward, 5' CAGACTTGCCCTCCAATTGATA 3' as reverse primers. PCR was carried out by using a Veriti™ 96-Well Thermal Cycler following the conditions for both primary and Nested amplifications: 94 °C for 5 min, 35 cycles of 1 min at 94 °C, 1.30 min at 60 °C, 2 min at 72 °C, followed by 35 cycles comprising 1 min at 94 °C, 1.30 min at 60 °C, 2 min at 72 °C and a final extension step of 10 min at 72 °C.

### 3.10 Human health risk assessment

To determine the risk of exposure among households, information on groundwater use was collected. Groundwater use was established by asking the respondents on their groundwater consumption practices.

A human health risk assessment was conducted by observation using a World Health Organization Risk assessment tool and Hazard Quotients (HQs) determined (Mendoza *et al.*, 2015).

$$HQ = \frac{I_{non-carcinogenic}}{RfD} \quad (\text{Mendoza } et al., 2015)$$

$$\text{where, } I = \text{Intake Rate } I = C \frac{CR \times EF \times ED}{BW \times AF}$$

C= average concentration of contaminant at exposure (mg/L in water)

CR=contact rate (L/day)

EF= exposure frequency (days per year)

ED= exposure duration (in years)

BW= body weight (Kg)

RfD= Reference Dose Factor (a ratio of the No-Observable Adverse Effect Level (NOAEL) divided by the Uncertainty Factor (UF)). This are values provided in tables.

HQ< 1; means that the water is safe and HQ>1; means that the water is unsafe. (Mendoza *et al.*, 2015)

### 3.11 Data processing and analysis

Three sets of data were collected for this study. Results were analysed using SPSS version 20 and STATA 14. The concentrations of antibiotics in water were determined and presented in µg/ L. Results for concentration of antibiotics were presented as means and ranges. Results of antibiotic sensitivity tests were expressed as a mean and median. Chi-square test of association was conducted between the concentration of antibiotics in groundwater and levels of antibiotic resistance, showing differences between the types of groundwater sources. A p-value of <0.05 was considered statistically significant.

Proportions were determined in the household surveys on antibiotic use and antibiotic disposal. Data were presented in tables and charts. Results of Key informant interviews were coded and analyzed manually according to emerging themes and narratives developed. Results of the Human Health Risk Assessment were displayed in a matrix and analyzed qualitatively. Based on the matrix, a decision of there being a risk of exposure was made and recommendations made.

### **3.12 Biases and confounders**

Measures to minimize the biases and confounders were considered as follows:

#### **Biases**

Information/ recall bias regarding antibiotic use. Heads of households were interviewed on antibiotic use among members of his/her family. The information given was not expected to be accurate but was overcome by ensuring clarity during the interview on the question and giving the respondent time to recall the occasions where members of the family used antibiotics.

#### **Confounders**

During analysis of water samples, it was anticipated that the levels of antibiotic residues of interest will be too low to detect. The most accurate dilution levels were employed to ensure the lowest limit of detection was achieved. The levels were however reported, and recommendation of different techniques was made.

Antibiotic residues may undergo degradation and/or undergo transformation limiting detection. This was an event beyond the project scope since a technique to dissociate the residues may be required.

### **3.13 Ethical consideration**

Ethical clearance to conduct research was obtained from three institutions as follows: The Health Research Ethic Committee of the university of the Witwatersrand (HREC. Protocol Number M190412); the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UoN-ERC. Ref No. P71910/2018); and the National Commission for Science, Technology, and Innovation (Ref No. NACOSTI/P/19/3232/28732). Each respondent gave informed consent to participate in the study. The confidentiality of the respondents was always maintained during and after the study. Their identities were not disclosed to anyone not in the research team. The health and water officials in Kisumu County were informed of any antibiotics found, microorganisms identified, and antibiotic resistance detected in any of the water for appropriate action to be taken.

### **3.14 Study limitations**

The present study was an academic study with restricted sources of funds, therefore a more complex study design with a larger sample was not used and therefore a descriptive study that focussed on only 5 informal settlements of Kisumu was used. However, the study's findings are applicable to and can be generalized to similar settings because informal settlements share characteristics that are not necessarily unique to a particular geographical area. A difference between informal settlements can be studied using a different study design. Cost of analysing water samples for antibiotics and sensitivity tests were high therefore, only a few water sources were sampled. A study that includes all groundwater sources in this informal settlement would give the true representation of magnitude of contamination of groundwater with antibiotics and antibiotic resistance. The study being cross-sectional, the data collected are not a reflection of the seasons of the year that may affect antibiotic concentrations in water. Therefore, another study design is recommended to incorporate the seasonal variation.

The study was implemented during the COVID-19 outbreak. This disoriented the original plans of collecting water samples and shipping to the proposed laboratory. For that reason, we sourced for a laboratory in Kenya where costs of analysing the samples was very high.

**CHAPTER FOUR:****RESULTS**

This section outlines the results of the study according to objectives.

**Assessment of sources of groundwater contamination with antibiotics in the community in five informal settlements in Kisumu, Kenya**

A total of 447 of households were visited in the five informal settlements. About 337 (75%) of the respondents were female and 110(25%) were male, majority of whom were below the age of 45 years (79% (n=354)). The number of households in each informal settlement were as follows: Obunga 24 (5.4%), Manyatta A 143 (32.1%), Manyatta B 89 (19.9%), Nyalenda A 96 (21.4%) and Nyalenda B 95 (21.2%).

**Table 7: Sociodemographic characteristics of the household heads in the informal settlements**

| Characteristic | Description | n (%)       |
|----------------|-------------|-------------|
| Sex            | Male        | 110 (25.0%) |
|                | Female      | 337(75.0%)  |
| Age            | 20-24       | 65 (14.5%)  |
|                | 25-34       | 173 (38.6%) |
|                | 35-44       | 115 (25.7%) |
|                | 45+         | 94 (21.2%)  |
|                |             |             |
| Settlement     | Obunga      | 24(5.4%)    |
|                | Manyatta A  | 143 (32.1%) |
|                | Manyatta B  | 89 (19.9%)  |
|                | Nyalenda A  | 96 (21.4%)  |
|                | Nyalenda B  | 95 (21.2%)  |
| HIV status     | Positive    | 21(4.7%)    |
|                | Negative    | 398 (88.8%) |

Most households had between one and four members 280 (62.5%), whereas in about 144 (32.1%) of the households there were between five and eight members and only 24 (5.4%) reported to have more than 9 members.

**Table 8: Number of people per household in the informal settlements**

| Number of people | Frequency (n) | Percent (%) |
|------------------|---------------|-------------|
| 1-4 members      | 280           | 62.5 %      |
| 5-8 members      | 144           | 32.1%       |
| 9> members       | 23            | 5.4%        |
| Total            | 447           | 100%        |

## Antibiotic use

Overall antibiotic use among households in the past one month of August 2019 (before the day of the interview) was at 43% (n=193). Among those consuming antibiotics, majority reported to have gotten a prescription after consultation with a healthcare practitioner 70% (n=137) while 29% (n=56) did not consult a doctor for the antibiotic prescription. Antibiotics were taken following a recommendation from friends 26% (n=13) and 76% (n=36) used antibiotics based on a previous experience. The level of awareness of health effects of consuming water that is contaminated with antibiotics was sought and found to be 35% (n=158), especially among households that used antibiotics p=0.003. Reasons for using the antibiotics varied, but the actual antibiotic used was not mentioned during the data collection.

Table 9 shows distribution of antibiotic source according to characteristics. A statistically significant difference was observed within the HIV status p=0.001 where a high proportion of the HIV positive respondents got a prescription form a medical practitioner (doctor) for antibiotics consumed. The significant difference observed (0.000) among the informal settlements was due to the reduced percentage of respondents obtaining a prescription for antibiotics from a doctor.

**Table 9: Percentage distribution of source of antibiotics against demographic characteristics.**

| Characteristics |            | Source of antibiotics             |                                |                                |                          |                            | p-value |
|-----------------|------------|-----------------------------------|--------------------------------|--------------------------------|--------------------------|----------------------------|---------|
|                 |            | Prescribed<br>by a doctor<br>n(%) | Advice from<br>friends<br>n(%) | Previous<br>experience<br>n(%) | Online<br>Search<br>n(%) | Other<br>(Chemist)<br>n(%) |         |
| Sex             | Male       | 37(77.1)                          | 4(40.0)                        | 8(80.0)                        | 0(0.0)                   | 2(1.7)                     | 0.408   |
|                 | Female     | 107(73.8)                         | 9(24.3)                        | 28(75.7)                       | 1(2.7)                   | 4(1.2)                     |         |
| HIV status      | Positive   | 14(87.5)                          | 0(0)                           | 2(100)                         | 0(0)                     | 0(0)                       | 0.001   |
|                 | Negative   | 121(75.2)                         | 12(31.6)                       | 29(76.3)                       | 1(2.6)                   | 5(1.3)                     |         |
| Settlements     | Obunga     | 9(81.8)                           | 0(0)                           | 1(50)                          | 0(0)                     | 1(4.2)                     | 0.000   |
|                 | Manyatta A | 62(81.6)                          | 0(0)                           | 13(100)                        | 0(0)                     | 0(0)                       |         |
|                 | Manyatta B | 24(64.9)                          | 9(69.2)                        | 11(84.6)                       | 0(0)                     | 1(1.1)                     |         |
|                 | Nyalenda A | 33(70.2)                          | 4(28.6)                        | 10(71.4)                       | 1(7.1)                   | 0(0)                       |         |
|                 | Nyalenda B | 16(72.7)                          | 0(0)                           | 1(20.0)                        | 0(0)                     | 4(4.2)                     |         |
| Age             | 20-24      | 17(70.8)                          | 5(71.4)                        | 2(28.6)                        | 0(0)                     | 3(4.6)                     | 0.086   |
|                 | 25-34      | 48(72.7)                          | 14(77.8)                       | 15(83.3)                       | 1(5.6)                   | 2(1.2)                     |         |
|                 | 35-44      | 39(73.6)                          | 9(69.2)                        | 13(100)                        | 0(0)                     | 0(0)                       |         |
|                 | 45+        | 40(80.0)                          | 6(66.7)                        | 6(66.7)                        | 0(0)                     | 1(1.1)                     |         |

The odds of using antibiotics were determined against the demographic characteristics at 95% confidence interval with no statistically significant difference (*table 10*). An increased odds of antibiotics use was noted among females than male participants with an odds ratio of 1.33. Among the HIV positive participants, the odds of using antibiotics were found to be lower than that of the negative participants (0.313). Participants aged 45 years and above had higher odds of using antibiotics as opposed to the younger heads of households (odds ratio= 1.54). The situation is however different among the settlements where odds of using antibiotics are reduced in only one informal settlement (Nyalenda B) (odds ratio =0.42).

**Table 10: Predictors of antibiotic use among household heads in the informal settlements**

| <b>Antibiotic use</b> | <b>Odds Ratio</b> | <b>Std. Err.</b> | <b>z</b> | <b>P&gt;z</b> | <b>95% Conf. interval</b> |
|-----------------------|-------------------|------------------|----------|---------------|---------------------------|
| <b>Sex</b>            |                   |                  |          |               |                           |
| Female                | 1.33              | 0.3105           | 1.24     | 0.215         | 0.845 - 2.105             |
| <b>HIV Status</b>     |                   |                  |          |               |                           |
| Positive              | 0.313             | 0.2097           | -1.73    | 0.083         | 0.084 - 1.163             |
| <b>Age</b>            |                   |                  |          |               |                           |
| 25-34 yrs.            | 0.96              | 0.302            | -0.12    | 0.908         | 0.522 - 1.782             |
| 35-44 yrs.            | 0.99              | 0.335            | -0.03    | 0.980         | 0.511 - 1.921             |
| 45+ yrs.              | 1.54              | 0.550            | 1.22     | 0.223         | 0.768 - 3.104             |
| <b>Settlements</b>    |                   |                  |          |               |                           |
| Manyatta              | 1.54              | 0.721            | 0.91     | 0.362         | 0.611 - 3.855             |
| Manyatta B            | 1.17              | 0.571            | 0.32     | 0.748         | 0.449 - 3.042             |
| Nyalenda A            | 1.61              | 0.778            | 0.98     | 0.325         | 0.623 - 4.153             |
| Nyalenda B            | 0.42              | 0.207            | -1.76    | 0.078         | 0.157 - 1.103             |
| <b>Cons</b>           | 2.263088          | 1.726156         | 1.07     | 0.284         | 0.507 - 10.091            |

### **Antibiotic disposal**

Respondents disposed antibiotics in various ways like in pit latrines 51.6% (n=16), throwing the remainder antibiotics in compost pits 16.1% (n=5), while others disposed by burning 6.5% (n=2). There are respondents however, who did not dispose the antibiotic dose not completed but kept for future use 87.1% (n=27). Analysis of completion of antibiotics showed that more females completed their antibiotic dose (117(36.3%)) as compared to men (39 (32.5%)). A statistically significant difference was observed in the HIV status (p=0.000), as most of the HIV positive respondents completed their antibiotic dose and a very small percentage of the HIV negative

respondents did not complete their antibiotic dose. A difference was also observed among the informal settlements ( $p=0.001$ ) where a lower proportion of respondents in Nyalenda B completed their antibiotic doses. Antibiotic completion increased with age but there was no significance difference among the age groups (*table 11*).

**Table 11: Distribution of completion of antibiotic dose by demographic characteristics**

| Characteristics | Description | Yes       | No        | p-value |
|-----------------|-------------|-----------|-----------|---------|
| Sex             | Male        | 39(32.5)  | 7(5.8)    | 0.647   |
|                 | Female      | 117(36.3) | 27(8.4)   |         |
| HIV status      | Positive    | 15(71.4)  | 1(4.8)    | 0.000   |
|                 | Negative    | 130(32.7) | 26(6.5)   |         |
| Settlements     | Manyatta A  | 63(43.8)  | 13(9.0)   | 0.001   |
|                 | Manyatta B  | 30(33.7)  | 6(6.7)    |         |
|                 | Nyalenda A  | 35(36.5)  | 11(11.5)  |         |
|                 | Nyalenda B  | 17 (17.9) | 4 (4.2)   |         |
|                 | Obunga      | 11 (45.8) | 0 (0)     |         |
|                 | 20-24       | 21 (32.3) | 2 (3.1)   |         |
| Age             | 25-34       | 55 (31.8) | 10 (5.8)  | 0.089   |
|                 | 35-44       | 40 (34.8) | 11 (9.6)  |         |
|                 | 45+         | 40 (42.6) | 11 (11.7) |         |
|                 |             |           |           |         |

### Ground water use

Almost all (99.8%  $n=446$ ) of the respondents used groundwater sources. Groundwater was used for various purposes in the households, like drinking (9% ( $n=39$ )), cooking (18% ( $n=82$ )), washing utensils (78% ( $n=350$ )), washing clothes (96% (421)) and cleaning house (95% (415)). A higher percentage of male respondents reported to be drinking groundwater 12.6%. going by HIV status, about 19% ( $n=4$ ) of respondents who reported to be HIV positive and 8.9% ( $n=35$ ) who reported to be HIV negative. used groundwater for drinking. Manyatta B and Nyalenda B settlements had more respondents who used groundwater for drinking. Groundwater use for drinking was found to increase with increased age. Water use is universal, therefore no differences observed.

**Table 12: Percentage distribution of groundwater use against sociodemographic characteristics**

|                    |            | Groundwater use |          |                  |                 |                | p-value |
|--------------------|------------|-----------------|----------|------------------|-----------------|----------------|---------|
| Characteristics    |            | Drinking        | Cooking  | Washing Utensils | Washing clothes | House cleaning |         |
|                    |            | n(%)            | n(%)     | n(%)             | n(%)            | n(%)           |         |
| <b>Sex</b>         | Male       | 15(12.6)        | 26(21.8) | 97(82.2)         | 112(94.9)       | 112(94.9)      | 0.261   |
|                    | Female     | 24(7.5)         | 56(17.5) | 253(79.1)        | 309(96.6)       | 303(95.3)      |         |
| <b>HIV status</b>  | Positive   | 4(19.0)         | 6(28.6)  | 18(85.7)         | 21(100)         | 20(95.2)       | 0.950   |
|                    | Negative   | 35(8.9)         | 71(18.0) | 312(79.2)        | 378(95.9)       | 376(95.9)      |         |
| <b>Settlements</b> | Obunga     | 3(12.5)         | 4(16.7)  | 15(62.5)         | 23(95.8)        | 23(95.8)       | 0.716   |
|                    | Manyatta A | 2(1.4)          | 14(9.9)  | 130(92.2)        | 141(100.0)      | 129(92.8)      |         |
|                    | Manyatta B | 12(13.5)        | 23(25.8) | 66(74.2)         | 83(93.3)        | 85(95.5)       |         |
|                    | Nyalenda A | 10(10.5)        | 19(20.0) | 76(80.0)         | 93(97.9)        | 91(95.8)       |         |
|                    | Nyalenda B | 13(13.7)        | 23(24.2) | 66(69.5)         | 87(91.6)        | 93(97.9)       |         |
| <b>Age</b>         | 20-24      | 2(3.1)          | 8(12.3)  | 47(72.3)         | 62(95.4)        | 59(90.8)       | 0.660   |
|                    | 25-34      | 12(7.1)         | 25(14.7) | 121(71.2)        | 161(94.7)       | 163(95.9)      |         |
|                    | 35-44      | 11(9.6)         | 27(23.5) | 103(89.6)        | 114(99.1)       | 107(94.7)      |         |
|                    | 45+        | 15(16.0)        | 23(24.5) | 81(87.1)         | 89(95.7)        | 91(97.8)       |         |

**Determination and comparison of concentrations of sulfamethoxazole, trimethoprim, and metronidazole in all groundwater sources in five informal settlements in Kisumu, Kenya.**

Different sources of water samples are provided in *table 13*. Shallow wells formed the majority of groundwater sources where water samples were drawn from.

**Table 13: Source of water sample**

| Water source | Frequency | Percent      |
|--------------|-----------|--------------|
| Shallow well | 39        | 79.6         |
| Spring       | 9         | 18.4         |
| Bore hole    | 1         | 2.0          |
| <b>Total</b> | <b>49</b> | <b>100.0</b> |

Physicochemical parameters of the water samples were measured using a HydroLab QUANTA multi parameter water quality meter. The physicochemical parameters measured included pH, water temperature and electric conductivity. The mean pH of the water samples was 7.3, with most of the samples having a pH below 7 (57.2%) (*table 14*).

**Table 14: Groundwater pH in the informal settlements**

| pH           | Frequency | Percent    |
|--------------|-----------|------------|
| 6.4 -6.9     | 28        | 57.2       |
| 7.0 -7.8     | 18        | 36.7       |
| 8.1 -8.3     | 3         | 6.1        |
| <b>Total</b> | <b>49</b> | <b>100</b> |

The water temperature ranged between 21.1 °C to 30.6 °C, with a mean of 25.7 °C. Electrical conductivity was also determined where 28.6% of the samples had conductivity value between 0-800  $\mu\text{S}/\text{cm}$ , whereas the majority had a value between 800-the maximum observed measurement of 2329  $\mu\text{S}/\text{cm}$  (71.4%,  $n = 35$ ) (*figure 7*). Electrical conductivity is a measure of the ability of water to pass electric current and is determined by concentration of electrolytes in the water where higher conductivity signify presence of more ions. Increase in water temperature is known to be related to increased conductivity but that is not the case in this study.

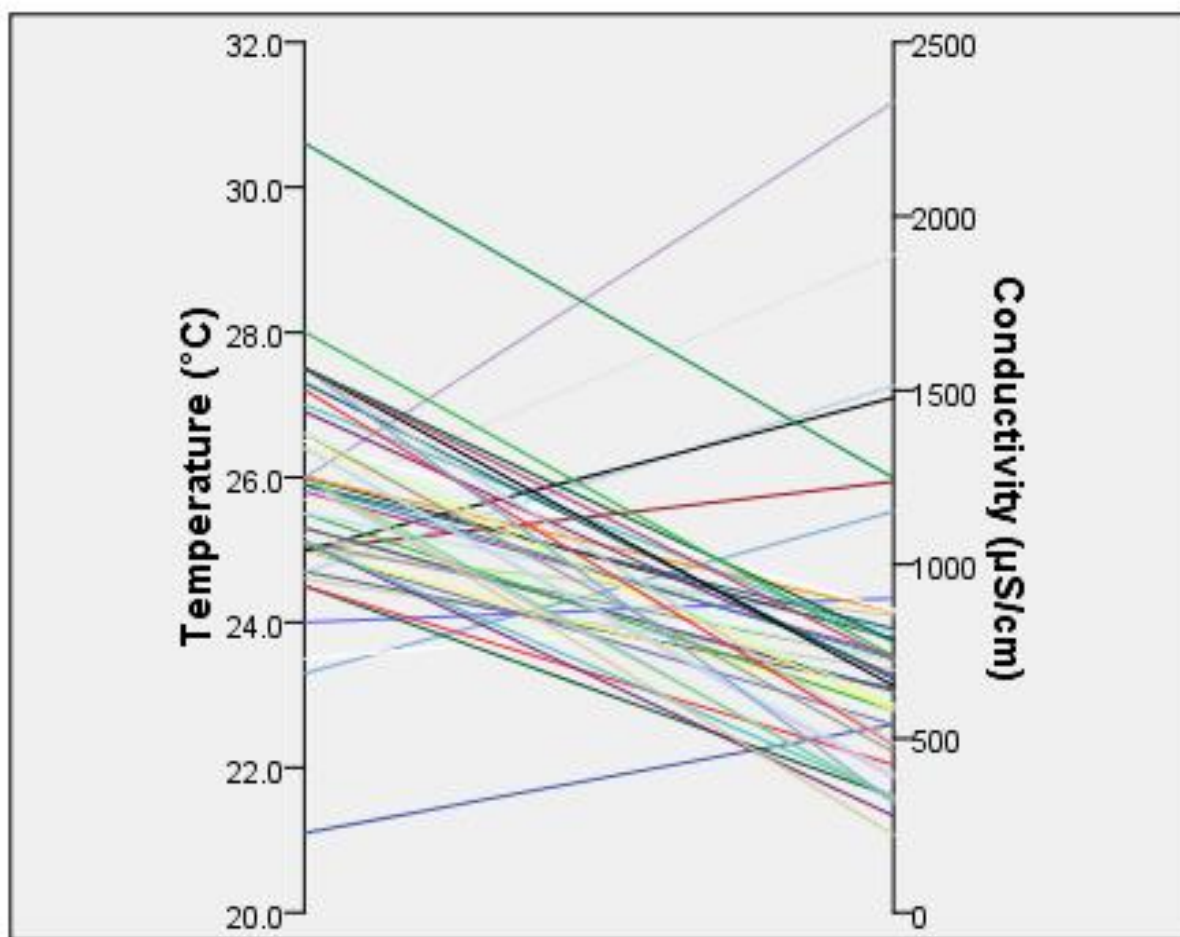


**Figure 7: Water conductivity ( $\mu\text{S}/\text{cm}$ ) and temperature ( $^{\circ}\text{C}$ )**

#### **Depth of groundwater source**

About 71% ( $n=35$ ) of the respondents were aware of the depth of the groundwater sources near them, where 85% ( $n=30$ ) were between 0.3 and 3.0 meters deep with the deepest being 27.5m which was a borehole.

**Table 15: Depth of groundwater source in the informal settlements**

| Depth of water source | Frequency (n=) | Percentage (%) |
|-----------------------|----------------|----------------|
| Below 1.0 meter       | 9              | 25.7           |
| 1.1-2.0 meters        | 12             | 34.3           |
| 2.1-3.0 meters        | 9              | 25.7           |
| 3.1-4.0 meters        | 3              | 8.6            |
| 4.1-5.0 meters        | 0              | 0.0            |
| 5.1 meters and above  | 2              | 5.7            |

**Concentration of antibiotics in groundwater sources**

Concentration of sulfamethoxazole, trimethoprim and metronidazole were determined in all 47 groundwater sources in the five informal settlements.

The reaction monitoring parameters for the three analytes were as shown in *table 16*.

**Table 16: The multiple reaction monitoring parameters.**

**(SMX = Sulfamethoxazole, TMP = Trimethoprim, MTZ = Metronidazole)**

| Target Compound | RT <sup>a</sup> | Precursor Ion [M + H] <sup>+</sup><br>(m/z) (CV) <sup>b</sup> | Quantifier Ion<br>(m/z) (CE) <sup>c</sup> | Qualifier Ion<br>(CE) |
|-----------------|-----------------|---------------------------------------------------------------|-------------------------------------------|-----------------------|
| SMX             | 4.74            | 254 (110)                                                     | 156 (12)                                  | 108 (24)              |
| TMP             | 4.24            | 291.1 (60)                                                    | 123.1 (30)                                | 80.9 (25)             |
| MTZ             | 3.32            | 172 (90)                                                      | 127.9 (10)                                | 82.1 (25)             |

<sup>a</sup>RT - retention time. <sup>b</sup>CV collision voltage. <sup>c</sup>CE collision energy

Method quantification results were as shown in *table 17*.

**Table 17: LC-MS/MS-ESI method quantification results.**

| API              | $r^2$  | % recovery | STDEV | RSD   | Repeatability | LOQ (ppb) |
|------------------|--------|------------|-------|-------|---------------|-----------|
| Metronidazole    | 0.98   |            |       |       |               | 53.03     |
| Spike 10 ppb     |        | 91.52      | 4.91  | 5.37  | 15.14         |           |
| Spike 50 ppb     |        | 90.56      | 4.63  | 5.11  | 14.27         |           |
| Spike 100ppb     |        | 97.12      | 5.24  | 5.4   | 16.15         |           |
| Trimethoprim     | 0.983  |            |       |       |               | 55.89     |
| Spike 10 ppb     |        | 112.7      | 1.69  | 1.5   | 5.19          |           |
| Spike 50 ppb     |        | 99.13      | 12.95 | 13.07 | 39.92         |           |
| Spike 100ppb     |        | 100.23     | 4.93  | 4.92  | 15.18         |           |
| Sulfamethoxazole | 0.9897 |            |       |       |               | 108.25    |
| Spike 10 ppb     |        | 106.83     | 4.62  | 4.32  | 14.24         |           |
| Spike 50 ppb     |        | 111.8      | 2.15  | 1.92  | 6.63          |           |
| Spike 100ppb     |        | 111.79     | 9.25  | 8.28  | 28.51         |           |

**API = Active pharmaceutical ingredient, ILIS = Isotopically labelled internal standard, LOQ = limit of quantification, SMX = sulfamethoxazole, TMP = trimethoprim, MTZ = Metronidazole**

Three (3) antibiotics (Sulfamethoxazole, Trimethoprim, and Metronidazole) were studied among 49 shallow wells in informal settlements of Kisumu County, Kenya in the ng/L range. The detection frequency for sulfamethoxazole in groundwater sources in the informal settlements of Kisumu was 14.3% (n=7). This was out of 49 groundwater samples, with concentrations ranging from no detection (nd) to 258.21 ng/L. All the water samples were from shallow wells. Trimethoprim and Metronidazole were however below the detection limit in groundwater. The detection frequency is presented in *table 18*.

**Table 18: Detection frequency of antibiotics in groundwater**

| Antibiotic       | Detection frequency (%)<br>(n= 49) | Range (ng/L) |
|------------------|------------------------------------|--------------|
| Sulfamethoxazole | 14.3                               | nd- 258.21   |
| Trimethoprim     | nd                                 |              |
| Metronidazole    | nd                                 |              |

nd: not detected

The average concentrations of Sulfamethoxazole are summarized in *table 19*. Sulfamethoxazole was detected in 7 out of 49 groundwater points with the levels ranging from a minimum concentration of  $12.7 \pm 1.1$  ng/L and a maximum concentration of  $258.2 \pm 27.4$  ng/L (SD=27.440). Trimethoprim and Metronidazole were not detected in any of the water samples collected from the groundwater sources. **Figure 9** represents the water samples (orange in color) on the map of the study area.

**Table 19: Concentration of Sulfamethoxazole in groundwater samples**

| Code  | Sample | SMX (ng/L) | SD   |
|-------|--------|------------|------|
| NyA4  | 1      | 12.7       | 1.1  |
| MyA10 | 6      | 25.4       | 10.6 |
| MyA8  | 9      | 33.6       | 9.5  |
| MyB4  | 10     | 258.2      | 27.4 |
| NyA10 | 14     | 24.6       | 1.1  |
| NyA2  | 18     | 16.4       | 0    |
| NyA1  | 20     | 29.9       | 8.4  |

SMX: sulfamethoxazole SD: standard deviation

Groundwater sources were mapped, water samples collected and analyzed for concentration of the antibiotics and antibiotic sensitivity test conducted against sulfamethoxazole as illustrated in *figure 8*.

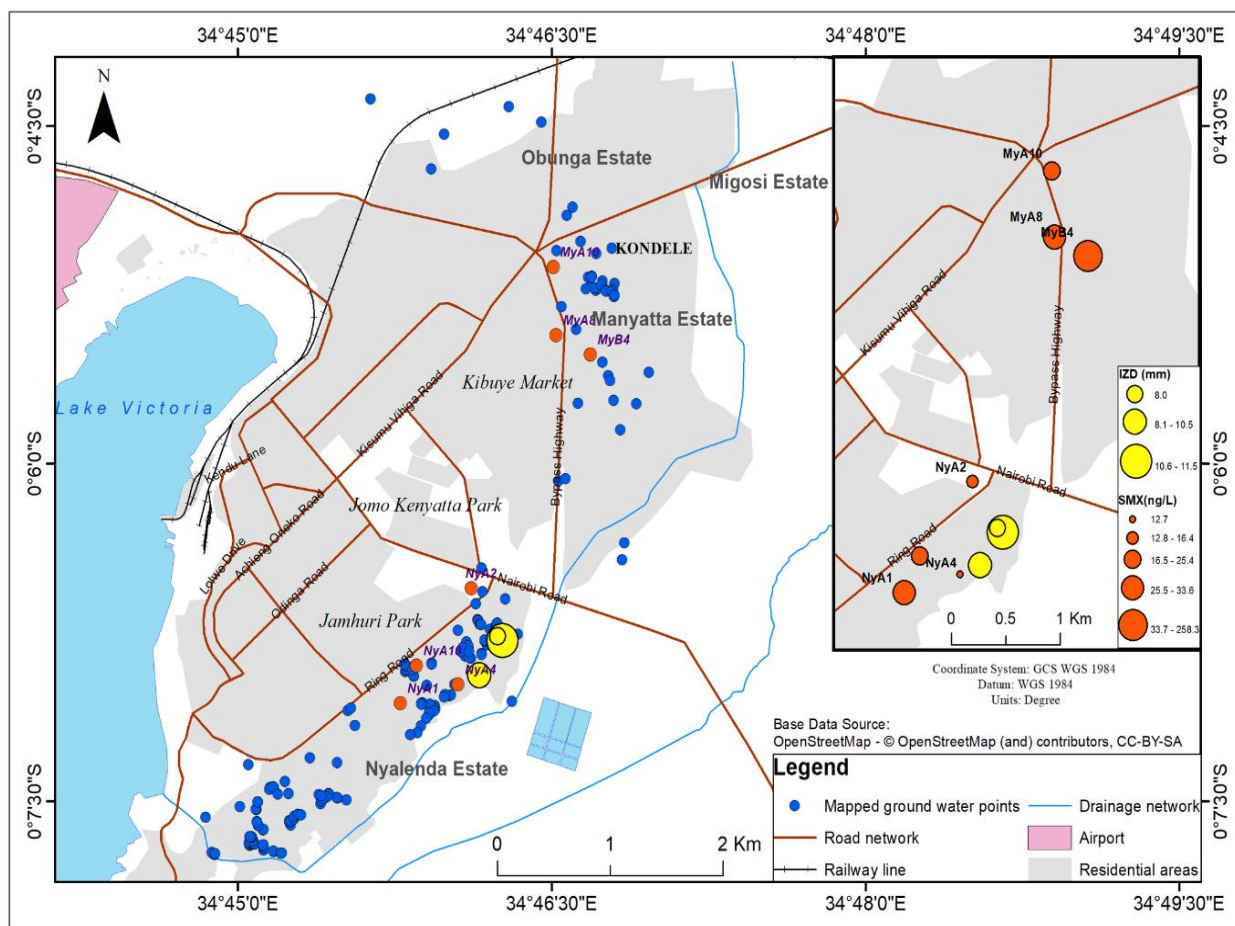


Figure 8: Map of study area showing the distribution of mapped groundwater points (blue), groundwater points where Sulfamethoxazole was detected (orange) and in groundwater sources where antibiotic resistant *E. coli* was detected (yellow).

**Determination of the presence of antibiotic resistant bacteria in water isolated from groundwater sources in five informal settlements in Kisumu, Kenya.**

**Isolation of *E. coli* and antibiotic sensitivity tests against Trimethoprim + Sulfamethoxazole**

A total of 49 groundwater sources were tested for *E. coli* and we found that 98% (n=48) were contaminated (Figure 9). The *E. coli* counts ranged from 16 to as high as 8,850; with a mean count of 1245 and a standard deviation (SD) of 1726.008 MPN/100ml. Antibiotic sensitivity tests revealed antibiotic resistance in three (3) groundwater points, representing about 6% of all the groundwater sources sampled. Water sources where antibiotic sulfamethoxazole was detected and the water points where resistant bacteria were detected were placed on the map of the study area as shown in **figure 8**.

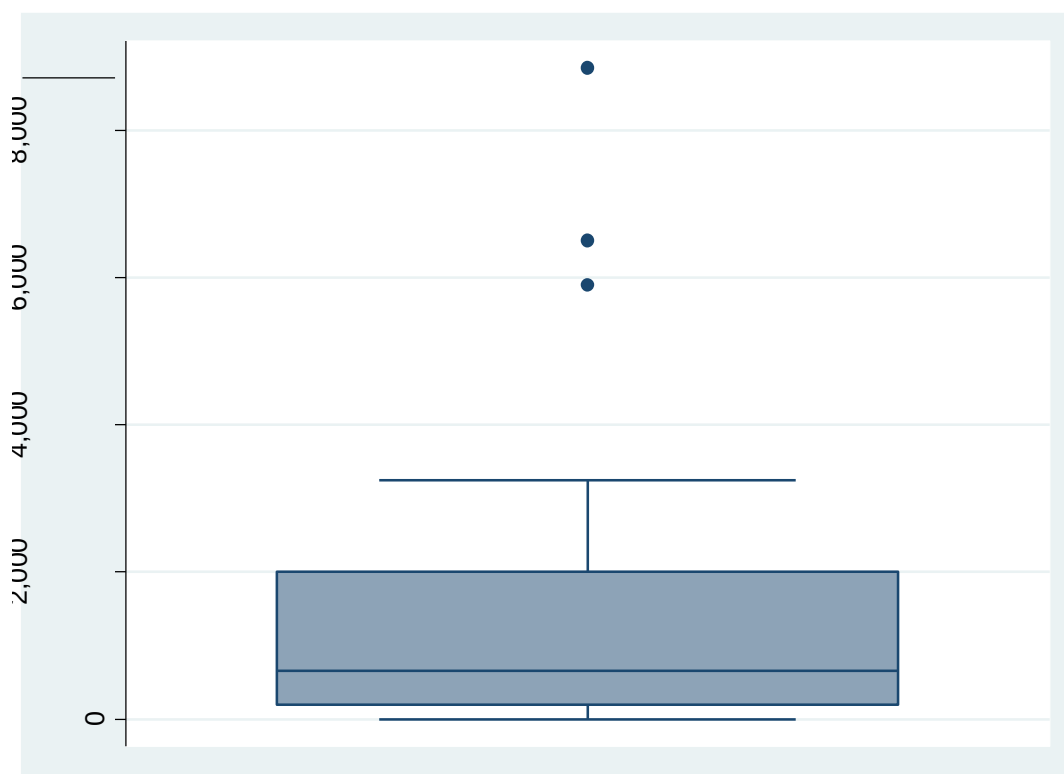


**Figure 9: Box plot showing *Escherichia coli* count in water samples (*E. coli* Count MPN/100ml water sample) collected from the groundwater sources in the informal settlements**

Antibiotic sensitivity test was conducted on *E. coli* isolates where inhibition zone diameters for 2 samples showed absolute resistance (IZD 10.5 mm and 8 mm), while one showed intermediate resistance (11.5 mm); with a standard deviation (SD) of 1.803. The zones of inhibition are shown in *table 20*.

**Table 20: Inhibition zones diameters for antibiotic sensitivity test on *E. coli* isolated from groundwater**

| Sample ID   | Zone of inhibition | Interpretation |
|-------------|--------------------|----------------|
| BS202130548 | 10.5 mm.           | Resistant      |
| BS202130537 | 08 mm              | Resistant      |
| BS202130526 | 11.5mm.            | Intermediate   |

### **Isolation of *Cryptosporidium parvum***

All samples were analysed for presence of *Cryptosporidium parvum*. Primary and nested PCR were conducted for the first 18 samples. The **Crypto - Primary PCR** detected 240 bp amplicon in sample 16 samples namely 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, and 18, thus they were positive *cryptosporidium parvum*, although 7, 8, 13 and 17 had a few amplicons of the required size. On the contrary, samples 5 and 15 were negative for amplicon size required thus negative for crypto (*figure 10*). The **Crypto Nested PCR**, for samples 1 to 14 however gave different results where all the samples were positive for Crypto species with amplicon size 240 bp (*figure 11*). The bands on the gel electrophoresis appear faint and unclear to visualize. This could be as a result of low protein concentration, incorrect electrophoresis conditions or problems with gel or buffer, due to insufficient quantity of DNA loaded on the gel.

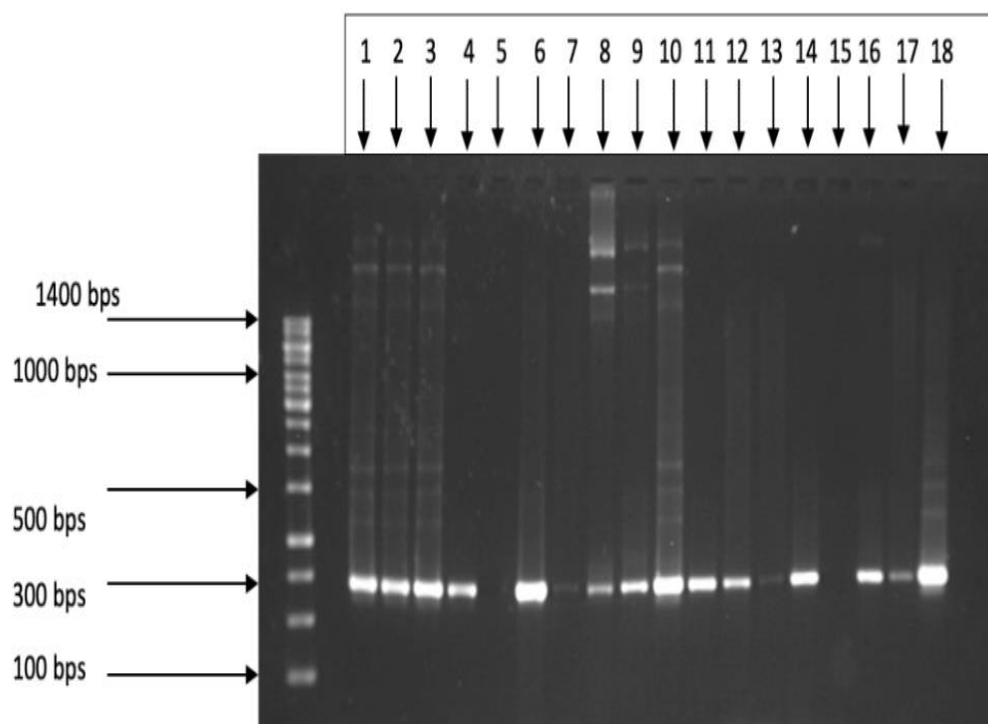


**Figure 10:** PCR product of *Cryptosporidium parvum* isolated from groundwater samples 1 to 18

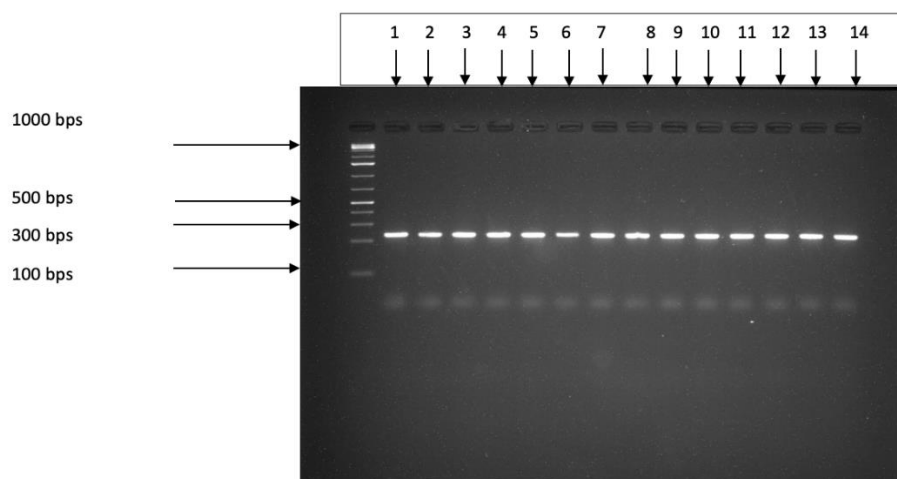


**Figure 11:** nested-PCR product of *Cryptosporidium parvum* isolated from groundwater samples 1 to 14

**An assessment of human health risk of exposure to antibiotics in the environment in five informal settlements in Kisumu, Kenya.**

Human Health risk assessment tool (World Health Organization) was used to qualitatively describe the antibiotic and the possible risks to the health of humans and the results are as shown in table 22.

**Table 22: Results of the human health risk of exposure to antibiotics present in water.**

| Question                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Expected output                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Hazard identification</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <p><b><u>Identity of the chemical</u></b></p> <p>Sulfamethoxazole was detected in groundwater. The antibiotic is commonly used in combination with trimethoprim to prevent and treat opportunistic infections such as urinary tract infection caused by <i>Escherichia coli</i> and Pneumocystis pneumonia caused by <i>Pneumocystis jirovecii</i> in HIV patients and the general population.</p> <p><b><u>Is the chemical potentially hazardous to humans?</u></b></p> <p>Contamination of groundwater sources with sulfamethoxazole has been evaluated in a variety of aquatic environments, with the predicted no effect concentration (PNEC) consistently indicating no risk of human exposure.</p> <p>However, there is a danger of resistance developing in bacteria that cause opportunistic infections in HIV patients, which poses a concern since it reduces the efficiency of infection therapy, potentially leading to prolonged hospitalization or death.</p> | <p><b><u>A clear identification of the chemical (antibiotics)</u></b></p> <p>Sulfamethoxazole is an ancient antibiotic in the sulphonamides class that is typically used in combination with trimethoprim. Sulfamethoxazole inhibits cytochrome P450 2C9 inhibitor. It completely inhibits dihydropteroate synthase, therefore blocking production of dihydropteroic acid required for the manufacture of folic acid, which is essential for bacterial growth.</p> <p>Sulfamethoxazole is an odourless yellow-white powder with a bitter in taste. It is slightly water (0.5g/L), and barely soluble in other solvents like ether and chloroform with a melting point of 167° C.</p> <p><b><u>Depiction of health hazards gotten from internationally available information and publications</u></b></p> <p>Antibiotics in drinking water have been linked to the spread of antibiotic-resistant bacteria. The bacteria <i>Escherichia coli</i>, which causes urinary tract infections, has been detected in water supplies. Furthermore, the bacteria have been found to be resistant to sulfamethoxazole. The immediate health risk of exposure to sulfamethoxazole in water is the failure of infection treatment, resulting in the usage of higher strength antibiotic preparations or death.</p> <p>Sulfamethoxazole is usually available in combination with trimethoprim, but only sulfamethoxazole was detected. Trimethoprim has a high distribution coefficient (pKa,</p> |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 7.12). Trimethoprim's large molecular weight, along with its poor solubility and mobility in water, are believed to play a role in its inaccessibility in groundwater. Another factor is the lower ratio of trimethoprim in the combination (5:1).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Hazard characterization</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <p><b><u>What is the chemical composition of that have the potential to cause adverse health effects?</u></b></p> <p>Sulfamethoxazole is a cytochrome P450 2C9 inhibitor that totally inhibits dihydropteroate synthase, preventing the generation of dihydropteroic acid, which is required for the formation of folic acid, which is required for bacterial growth. Antibiotic resistance occurs in the environment, including groundwater, rendering antibiotics useless for treating human illnesses.</p> <p><b><u>Is there guidance or guideline values from international organizations exist for the chemical?</u></b></p> <p>There are no known levels of antibiotic that cause environmentally induced resistance</p> <p><b><u>What assumptions on exposure and dose are described by the available guidance/ guideline values for the chemical?</u></b></p> <p>Because groundwater is utilized regularly to supplement water shortages, it is assumed that the exposure occurs continuously.</p> <p><b><u>Do the assumptions reflect conditions specific to the local population?</u></b></p> <p><b>Yes.</b> This is an informal settlement where water is scarce therefore, residents supplement water for domestic use with the readily available groundwater that has been found to be polluted with the antibiotic sulfamethoxazole.</p> | <p><b><u>Qualitative or quantitative description of the properties of the chemical with potential to cause adverse health effects.</u></b></p> <p>The antibiotic has been detected in water in various settings where the predicted no effect concentration (PNEC) has consistently suggested that there is not risk of human exposure. The quantity of antibiotics in water that trigger development of resistance in microbes is unknown.</p> <p><b><u>Guidance values (rates or concentrations) for chemical obtained from internationally available resources</u></b></p> <p>Sulfamethoxazole has been detected in water in various settings where the predicted no effect concentration (PNEC) has continuously indicated no risk of exposure among humans.</p> <p>The level of antibiotics required to trigger resistance in bacteria is also not documented</p> <p><b><u>List of assumptions about contact rates, absorption</u></b></p> <p>Safe water for domestic use is scarce and people living in informal settlements supplement their daily water needs with the readily available groundwater that is used without being subjected to any form of treatment.</p> <p>It is assumed that they used the water for drinking contrary to the reported use.</p> <p><b><u>A reference value reflecting exposure and dose parameters specific to the given demographics</u></b></p> <p>Reference values for the specific populations are not available since concentrations that trigger resistance are unknown</p> |

|                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Exposure assessment</b>                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <p><b><u>What are the possible ways of people coming into contact with the chemical?</u></b></p> <p>People are exposed to the antibiotic via ingestion of groundwater.</p> <p><b><u>What measure of the exposure is required for characterizing health risks?</u></b></p> <p>There is no known limit of exposure that causes antibiotic resistance in the environment</p> | <p><b><u>Qualitative description of relevant media/ routes of exposure</u></b></p> <p>Antibiotics detected in water sources are the result of poor antibiotic usage and disposal practices, as well as incomplete metabolism in the human body, which results in antibiotics being excreted in urine or faeces either unchanged or as active ingredients. They have been found to contaminate groundwater by leaching from sanitation facilities like pit latrines, septic tanks and waste disposal sites where the aquifer is easily accessible and sensitive to rainfall recharge in the study area.</p> <p>Populations are exposed to the antibiotics in water through ingestion.</p> <p><b><u>Determination from given guidance values of whether an exposure rate is needed to perform the risk characterization</u></b></p> <p>Sulfamethoxazole concentrations in groundwater ranged from <math>12.7 \pm 1.1 \text{ ng/L}</math> to <math>258.2 \pm 27.4 \text{ ng/L}</math> (Mean= 57.26; SD=27.440). The mean concentration is lower than that reported by Mendoza <i>et al</i> 2015 where a Hazard Quotient was computed to determine risk of exposure to antibiotics in the environment.</p> |
| <b>Risk characterization</b>                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <p><b><u>What is the comparison of the estimated exposure with guidance/ guideline values for the chemical?</u></b></p> <p>There is no known limit to guide the process of characterization since exposure limits that cause antibiotic resistance in the environment have not been documented.</p>                                                                       | <p><b><u>A quantitative or qualitative non-risk statement (e.g., Hazard Quotient)</u></b></p> <p>The hazard quotient computed for the study was found to be equal to 0 (zero); Zero is less than 1, therefore, no risk of exposure to sulfamethoxazole in water.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

### Hazard quotients (HQs) determination

(Mendoza et al., 2015; World Health Organization, 2003)

The hazard quotient was computed as follows: -

$$HQ = \frac{I_{non-carcinogenic}}{RfD} \quad (\text{Mendoza et al., 2015})$$

Where, I= Intake Rate  $I = C \frac{CR \times EF \times ED}{BW \times AF} = 0.000005800360490$ ; where

C= average concentration of contaminant at exposure (mg/L in water) = **0.0002582mg/L**

CR=contact rate (L/day) = **1.5** (based on receptor characteristics as recommended)

EF= exposure frequency (days per year) = **365**

ED= exposure duration (in years) = Not applicable since the number of years of exposure varied

BW= body weight (Kg)= **66.5kg** (average body weight)

RfD= Reference Dose Factor (a ratio of the No-Observable Adverse Effect Level (NOAEL) divided by the Uncertainty Factor (UF)). This are values provided in tables.

HQ< 1; means that the water is safe and HQ>1; means that the water is unsafe. (Mendoza et al., 2015)

The hazard quotient was found to be 0 (zero); meaning that there is no risk of exposure to sulfamethoxazole in groundwater.

**CHAPTER FIVE:****DISCUSSION****Introduction**

Water is a necessary component of life that must be protected in order to protect the health of humans, animals, and the environment. Contamination of groundwater supplies by substances such as antibiotics is a silent pandemic that, if not treated, has the potential to intensify, leading to development of environmentally induced antibiotic resistance.

This study looked into antibiotic contamination of community groundwater sources in informal settlements. In this section, the study findings will be discussed and compared with similar studies in the region and globally. The section is organized based on the study's objectives. A portion of the research has been published in peer-reviewed publications and policy papers for wider distribution.

**Sources of groundwater contamination with antibiotics in the community in five informal settlements in Kisumu, Kenya**

Objective one assessed sources of groundwater contamination. Towards this, variables that were collected among the household heads in the study cite included their demographic characteristics, groundwater use, antibiotic disposal practices, awareness of health effects as a result of consuming water that is contaminated with antibiotics.

The following manuscript was published as:

**Citation: Karimi, K.J.; Ahmad, A.; Duse, A.; Mwanthi, M.; Ayah, R. Prevalence of Antibiotic Use and Disposal at Household Level in Informal Settlements of Kisumu, Kenya. *Int. J. Environ. Res. Public Health* 2023, 20, 287. <https://doi.org/10.3390/ijerph20010287>**

The discovery of antibiotics for the treatment of infections and prophylaxis in human and animal infections, but has advantages and disadvantages in that the benefits are maximum when properly used but on the contrary harmful to the users if not properly used (WHO, 2018) or disposed, and in equal measure harmful to the environment (Kraemer, Ramachandran, & Perron, 2019; Larsson & Flach, 2022). The effect on any of the components of the one-health triad complicates treatment of infections when antibiotic resistance develops; a problem that become more complex when the resistance is environmentally induced since the role played by the environment in spreading

resistance is difficult to quantify and reduce (Bengtsson-palme, Jonsson, & Heß, 2021; Kraemer *et al.*, 2019; Larsson & Flach, 2022).

Groundwater is a naturally occurring source that is accessible through shallow aquifer and used for domestic purposes, agricultural and industrial globally for it is estimated that one third of the population worldwide rely on groundwater for drinking. Majority of residents in the informal settlement studied reported to have used groundwater for other domestic purposes which included cooking, drinking, washing utensils, washing clothes and cleaning the houses (Okotto *et al.*, 2015). Groundwater use has been reported in various settings as reported by a multi-country review of the Southeast Asia and the Pacific on the current reliance of groundwater, where an average of 62% (17- 93%) urban populations and 60% (22-95%) rural populations relied on groundwater for drinking (Carrard, Foster, & Willetts, 2019). This reliance on groundwater for domestic use is as a result of shortages occasioned by limited supply by the conventional water sources by municipalities. A baseline study on groundwater reliance found a higher rate (79%) of use for drinking at household level and the main concern is that the water may be polluted by unsafe sanitation practices, coupled with the recent challenges brought about by effects of climate change (Carrard, Foster, & Juliet, 2019). Groundwater provides for nearly 50% of water used in urban areas, therefore protecting from potential pollutants that are harmful to health of water is mostly important (Li, 2017). The quality of the resource is however under threat due to the rising population that encourage urbanization and proliferation of informal settlements (United Nations, 2015), industrialization, current agricultural practices and climate change (Li & Srinivasamoorthy, 2021). The groundwater sources have exhibited resilience to drought, and provides they are well managed and protected, they are well positioned to augment water security for a wide range of users sustainably. Serious water supply challenged experienced in major cities of the world like Cape town, Sao Pauli and Chennai are a lesson to cities, highlighting vulnerability of surface water sources and the failure to incorporate groundwater as a supplementary water source to enhance waste security (Foster, 2022).

Contaminants like heavy metals, pesticides, nanoparticles, micro plastics and emerging contaminants like pharmaceutical compounds have been detected in groundwater over the past decades and they have been found to be harmful to human health, the environment and sustainable, socio-economic development (Li, 2020; Li & Wu, 2019). Residents of informal settlements are likely to be exposed to contaminants like antibiotics in water and risk of antibiotic resistance. Groundwater sources in this study were contaminated with the antibiotic sulfamethoxazole which

is available in combination with trimethoprim and is recommended for prevention and treatment of infections like urinary tract infections caused by *Escherichia coli* and Pneumocystis pneumonia cause by *Pneumocystis jirovecii* among HIV/AIDS patients and also in the general population (World Health Organization, 2016) and this exposes the populations, lowering the effectiveness of treatment.

Prevalence of antibiotic use in the study area was reported to be 43%, which differs from results of an entry/exit survey conducted in an urban informal settlement which reported up to 87% prevalence of antibiotic use (Omulo *et al.*, 2017). This has been attributed to the fact that the densely populated informal settlements suffer sanitation challenges that facilitate disease transmission thus encouraging antibiotic use (Omulo *et al.*, 2021). In a study justifying the role of environmental risk factors in facilitating carriage of antimicrobial resistant bacteria in a high density urban informal settlement, prevalence of self-reported antibiotic use was found to be even higher at 97% (Omulo *et al.*, 2021). The high prevalence of antibiotic use is a pointer to possible contamination of the environment that include groundwater with antibiotic and antibiotic resistant bacteria. This is needs to be monitored, especially in the current study site where the prevalence of HVI/AIDS is also high. If antibiotics used for the prevention and treatment of various bacterial infections are continuously detected in the environment, and groundwater in particular, there will be a problem of reducing related morbidity and mortality, especially among people with compromised immunity.

Overuse or misuse of antibiotics is essential in mitigating the risk of development of antibiotic resistance. This however requires healthcare professional and the general public to be informed of the importance of completing the dose as prescribed (Darwin & Gorton, 2017). In this study, respondents did not necessarily acquire a prescription for the antibiotics used and did not get rid of them but reported to have kept them for use the next time they were sick. Some disposed of the antibiotics by throwing in pit latrines or compost pit whereas others set the remainder antibiotics on fire. The disposal practices have however been found to contaminate the environment (Anwar *et al.*, 2020; Bashaar *et al.*, 2017; WHO, 2007) which include water sources.

Population increase has led to proliferation of informal settlements where people experience deplorable living and environmental conditions (Ben C. Arimah (UN-HABITAT), 2017). In these settlements, demand for housing water and sanitation facilities has in turn led to an increase in number of households, shallow wells, and density of unimproved sanitation facilities between the year 2009 and 2015 (Okotto *et al.*, 2015). Anthropogenic activities have been found to contaminate

groundwater, with a study in China reporting drastic deterioration of the deep and shallow aquifers and up to 85% of the groundwater declared poor or very poor (Han, Currell, & Cao, 2016). The aquifer within the study area is easily accessible and prone to contamination from various sources that include poor drainage system, low quality sanitation facilities in close proximity that may enable cross-contamination (Okotto-okotto *et al.*, 2015; Othoo *et al.*, 2020). Therefore, ensuring safety of groundwater is an important step towards sustainable development for any nation (Li & Srinivasamoorthy, 2021). A marginal increase in population has been noted over a period of ten years in the current study site, where the number of households increased between the year 2009 and 2019 (National Bureau of Statistics, 2019).

### **Concentration levels of sulfamethoxazole, trimethoprim, and metronidazole in all groundwater sources in five informal settlements in Kisumu, Kenya**

In this objective, we determined the concentration levels of sulfamethoxazole, trimethoprim, and metronidazole in groundwater samples. Sulfamethoxazole and trimethoprim are a combination of antibiotics used in the prevention and treatment of *Escherichia coli* and *Pneumocytis pneumonia* infections mostly among HIV/AIDS patients and in the general population and on the other hand metronidazole is used by the same population for the treatment of parasitic infections like *Cryptosporidium parvum*, which is a major cause of diarrhea among HIV/AIDS patients.

Evidence of contamination of water sources with emerging contaminants like pharmaceutical compounds has been demonstrated, but there is still little research among groundwater sources (Lapworth *et al.*, 2012; Sui *et al.*, 2015), particularly in developing countries. Sources of these antibiotics have been found to be human sewage systems (Dolejska *et al.*, 2011), intensive animal farming as well as pollution from pharmaceutical manufacture (Larsson, 2014b). A review on emerging pollutants in water environment reported continuous occurrence of emerging contaminants, but also noted that there is need for fast and efficient detection of the contaminants (Tang *et al.*, 2019). In this study, only sulfamethoxazole was detected in groundwater sources, at a detection frequency of 14.3% (n=7) and levels ranging from minimum concentration of  $12.7 \pm 1.1 \text{ ng/L}$  and a maximum concentration of  $258.2 \pm 27.4 \text{ ng/L}$  ( $SD=27.440$ ). This is consistent with a study conducted in the same study area that targeted a group of pharmaceuticals in one groundwater source with concentrations between  $5\text{-}50 \mu\text{g/L}^{-1}$  (K'oreje *et al.*, 2016). Sulfamethoxazole and trimethoprim have been reported to be the most detected antibiotics in all

water matrices globally, and unprotected groundwater sources are the most susceptible to contamination with antibiotics from surface water and wastewater. The detection frequency for SMX corroborates with findings of a review where concentrations of  $2.53 \mu\text{g/L}^{-1}$  were reported in an African aquatic environment. Sulfamethoxazole was also reported to be the most frequently detected antibiotic in groundwater where forest land was irrigated with wastewater ( $5 \mu\text{g/L}^{-1}$ ), but much higher concentrations were recorded where agricultural land was irrigated with wastewater ( $500 \mu\text{g/L}^{-1}$ ) in the USA.

Fate of antibiotics in the environment that include water is unknown, thus very important to study. Trimethoprim, on the other hand was not detected in any of the groundwater sources, and this was attributed to the high distribution coefficient ( $\text{pK}_a$ , 7.12) (Franklin *et al.*, 2022). A study in Lusaka Zambia detected trimethoprim at a lowered pH of between 4.02 to 5.56, which was considered to be the optimum sorption range and maximum concentrations of  $140\text{ng/L}$  (Ngumba *et al.*, 2020). The water pH in this study was between 6.4 and 8.3, which is higher than that of the Zambian study. Trimethoprim is however generally not present in groundwater due to its higher molecular weight (290.332 Da) which renders it less mobile as well as its weak solubility in water (Viana *et al.*, 2021), in fact, only 2 out of 29 groundwater sources were positive for TMP. Furthermore, the unavailability of TMP can be explained by the fact that it is used in combination with Sulfamethoxazole BP in a ratio of 1:5, each tablet containing 80mg trimethoprim and 400mg sulfamethoxazole (WHO, 2016), making SMX five times higher in water than TMP. Sorption qualities of TMP could also be explained by the hydrogeology of the study area where the soil is predominantly clayey with high porosity and low permeability (Kanoti *et al.*, 2019). The disparity in occurrence of the antibiotics in water could also be affected by the physicochemical properties of groundwater in the study area. However, the fate of TMP in groundwater should be cautiously investigated since there could be a danger of development of transformational compounds that could be more difficult to detect as well as development of resistant pathogens in the environment is a possible event (Larsson & Flach, 2022) which if not addressed has a potential to get out of hand. It is vital to have a solid understanding of the fate of pharmaceutical pollutants, particularly antibiotics from sanitation facilities such as pit latrines and septic tanks that share the subsurface with shallow aquifers. This is due to the reliance on groundwater for domestic use and the related risk of antibiotic resistance development. As a result, it is critical to begin conversations about antibiotic use and disposal.

Metronidazole is also an important antibiotic used for treatment of the protozoan parasites of the genus *Cryptosporidium* (Centers for Disease Control and Prevention (CDC), 2022), which commonly cause chronic diarrhea in persons with HIV/AIDS in developing countries (Dandachi D. *et al.*, 2021). Metronidazole was, not detected in the groundwater samples collected, contrary to findings in the same study area where metronidazole was detected in two shallow wells of an informal settlement in the study area. Lack of detection of Metronidazole in groundwater could be attributed to its high solubility (Cheng *et al.*, 2013; Nolasco *et al.*, 2013) in water which could result in the molecule being transported at a higher rate in water. Metronidazole is however not biodegradable in water, but coupling this property with its high solubility may lead to accumulation in aquatic environments, therefore important to monitor to protect against toxicity and possible development of resistance in the environment.

The Kisumu County government is aware that people in the informal settlements supplement their water needs with groundwater because water is a limited commodity. The officials in charge of water, sanitation, and health were astonished to find not just drugs in the groundwater, but also antibiotic resistance microorganisms. Officials from the Kenya Water Institute and the Kisumu Water and Sanitation Company were unaware that the presence of antibiotics in the environment leads to the development of antibiotic resistance, and they wanted to know where the antibiotics came from and what causes antibiotic resistance in *E. coli*. It was even more surprising to hear that *E. coli* resistant to sulfamethoxazole had been isolated from groundwater sources where the antibiotics had not been detected. There was general agreement that groundwater sources containing resistant *E. coli* should be mapped, and resistance triggers should be discovered and avoided in order to avoid a broader scale of resistance.

### **Presence of antibiotic resistant bacteria in water isolated from groundwater sources in five informal settlements in Kisumu, Kenya**

In this objective, we isolated *E. coli* and *C. parvum* from groundwater and tested the *E. coli* for resistance against Sulfamethoxazole. We did not conduct a sensitivity test for trimethoprim since the study was sequential and the moment, we did not detect trimethoprim in the groundwater, only towards SMX was determined.

### **Occurrence of *Escherichia coli* in groundwater sources and antibiotic resistance**

Water is unsafe for consumption if contaminated with the pathogens and if ingested through exposure in the environment, the pathogens pose health risks to humans and animals, thus

contributing to high morbidity and mortality globally (WHO, 2017). These pathogens are a source of contamination as they get to the water through leaching or other mean like untreated sewage (Bennett *et al.*, 2018). *E. coli* is a faecal microbe that inhabit the intestines of endotherms as the primary habitat as well as the environment that include water as secondary habitat (Petersen & Hubbart, 2020). The habitats vary in physicochemical conditions and nutrient availability (Savageau, 1983). For example, the primary habitat temperatures can be constant (anaerobic environment) while the secondary habitat vary, ranging from as low as 0 °C to about 18 °C or higher and include deep soil, sediments, and water (Savageau, 1983). This study determined factors that influence occurrence and survival of *E. coli* in their secondary habitats (environment) and are used to quantitatively predict changes in levels of *E. coli* in the environment which included water temperature, pH, electrical conductivity, and depth of groundwater sources. The water temperature ranged between 21.1 °C and 30.6 °C which is within the optimum temperature within which the bacteria thrive. This is consistent with a study by Jones *et al.*, 2004 which concluded that the temperature of between 0 ° and 47 °C and presence of nutrients form an excellent environment for survival of *E. coli*; with the growth limit of 7 °C and tolerance range of between -20 °C and 66 °C being a demonstration of how temperature alters bacterial growth (Jones *et al.*, 2004). Generally, growth and survival of *E. coli* is directly affected by soil temperature fluctuations with growth increasing as the temperature increases at between 15 °C and 37 °C, thus temperature is a vital environmental factor influencing *E. coli* survival in its secondary habitat (Ishii *et al.*, 2006; Mcquestin *et al.*, 2009; Shii *et al.*, 2010). Growth of *E. coli* was inhibited by lowered pH but fluctuated with increased temperature (Petersen & Hubbart, 2020). In the current study, however, the pH range was between 6.4 and 8.3, which could be the reason as to why occurrence of *E. coli* was at 98% (n=48). This study did not evaluate land use practices and hydrologic conditions like precipitation, and subsequent run-off, but studies have shown increased *E. coli* count with increased land use and occurrence of the hydrologic conditions stated earlier. On the other hand, the effect of exposure to sunlight was found to reduce *E. coli* count.

Electrical conductivity is a measure of saltiness of the water and is measured on a scale from 0 to 50,000 sS/cm. It is measured in microsiemens per centimeter (uS/cm). Fresh water is usually between 0 and 1,500 uS/cm and typical sea water has a conductivity value of about 50,00 uS/cm. Increased saltiness can cause problems for aquatic life forms and complicate use in humans. Increased water salinity impairs *E. coli* growth as demonstrated by Stahl *et al.* 2016, where *E. coli* growth was inhibited by increased salinity (Stahl *et al.*, 2016). The findings are consistent with

those of this study where we reported salinity of between 0 and 2,500 uS/cm. A significant association was observed between *E. coli* count and electric conductivity of groundwater  $p=0.039$ . Water with conductivity between 0 and 800 is good for drinking for humans (provided there is no organic pollution and suspended clay material. It is generally good for irrigation but above 300 uS/cm care must be taken especially for overhead sprinklers which may result in scorch o some salt sensitive parts of the plan. On the other hand, conductivity of between 800 and 2,500 uS/cm can be consumed by humans, but most would prefer water with less conductivity. Special management is needed when used for irrigation. It is suitable for use by livestock.

*Escherichia coli* are generally harmless organisms that are members of the gut flora in both humans and warm-blooded animals. They are also known to be an opportunistic pathogen and have been linked to intestinal illness, urinary tract infections, newborn meningitis, and gastroenteritis (Braz *et al.*, 2020; Kaper *et al.*, 2004; Köhler & Dobrindt, 2011). Contamination of water supplies by coliforms like *Escherichia coli* mostly is as a result of lack of adequate sanitation, which is a common phenomenon in informal settlements (Gqomfa *et al.*, 2022; Opisa *et al.*, 2012). The bacteria are reported as an indicator of fecal contamination in the assessment of water quality deterioration, which is a public health concern.

*Escherichia coli* is present in a variety of water sources, including those used for drinking (Saxena, Kaushik, & Krishna, 2015). Consistent with the findings of this study, *E. coli* was identified in groundwater in South Africa, and the contamination was linked to informal settlements and environmental deterioration (Gqomfa *et al.*, 2022). A study in Bangladesh found *E. coli* and fecal coliforms in drinking water sources and household point of use, which was attributed primarily to inadequate hygiene (Mahmud *et al.*, 2019). This study's findings are also consistent with those of a 2012 study conducted in the same Kisumu informal settlements, which found that up to 95% (n=76) of the sampled water points were highly contaminated with *E. coli*; all water samples from unprotected wells and 92.6% (n=25) of the water from protected wells were positive for *E.coli*, with low densities observed from dams and boreholes (Opisa *et al.*, 2012). A research conducted in Kinsasha's sub-rural areas discovered high levels of *E.coli* contamination in groundwater and recreational water, which was connected to inadequate sanitation (Kayembe, Thevenon, La, & Sivalingam, 2018). A study of groundwater quality based on presence of *E. coli* in densely populated areas of South Jakarta reported a weak association between presence of *E. coli* in groundwater and proximity to septic tanks but concluded that *E. coli* in groundwater is largely

influenced by water flow based on the soil topology of the region, which is closely related to hydrogeology of the region (Y Rohmah, A Rinanti, 2018).

There is rising worry about the prevalence of antibiotic-resistant bacteria and antibiotic-resistant genes in aquatic environments, with industries, agriculture, and domestic antibiotic usage having been identified as sources of aquatic pollution. (Marti *et al.*, 2014; Pereira *et al.*, 2021). Studies focusing on antibiotic resistance have concentrated in aquatic environments heavily contaminated with antibiotics as a result of activities like wastewater treatment and anthropogenic activities such as farming (Middleton & Salierno, 2013; Zhang *et al.*, 2015). Presence and spread of antibiotic resistance in natural water used for irrigation, aquaculture, recreation, drinking and other domestic purposes are ignored whereas the importance to human life is well known. The direct or indirect use of the water contaminated by antibiotic resistant bacteria could have an effect on the health of human beings, animals and contaminate the environment (Chen, C., Li, J., Chen, P., Ding, R., Zhang, P., & Li, 2014; Q. L. Chen *et al.*, 2017). Bacteria resistant to at least one antibiotic was isolated from up to 80% of groundwater sources, and resistant genes identified, and a recommendation on further research to understand triggers of resistance in the environment recommended (Andrade *et al.*, 2020). A study in Northern Tanzania isolated *E. coli* that was resistant to one or more antibiotics, and to be specific, resistance against ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim was higher than that of other antibiotics (Lyimo *et al.*, 2016). Antibiotic resistant *E. coli* was isolated in drinking water among households in Cajamarca Peru, where sulfamethoxazole-trimethoprim resistance was at 21.4%; necessitating analysis of environments for contaminants and if the sources of the contamination are as a result of antibiotic misuse or improper disposal at household level (Larson *et al.*, 2019).

In a study by Omulo *et al* 2021, *E. coli* isolated from stool samples, drinking water samples and hand swabs were found to be resistant to five antibiotics that included Sulfamethoxazole (81%) and Trimethoprim (80%). The drinking water samples were not specifically collected from groundwater sources, but the fact that resistant bacteria are detectable in water samples in informal settlement should be of concern since informal settlements are comparable in terms of shared characteristics. In a study conducted in Japan, *E. coli* isolated from the urinary tract was found to be resistant to cotrimoxazole (sulfamethoxazole and trimethoprim), a trend that has gradually increased since 1970's (Satoshi *et al.*, 2002). Epidemiological studies found the rate of cotrimoxazole resistance *E. Coli* isolated from uncomplicated UTis to be about 15.7% in European countries, with Portugal and Spain reporting about 34.8% (Kahlmeter, 2015) and 18% in America

(Satoshi et al., 2002), whereas in Japan, prevalence in hospital isolates being about 4.5%, a rate which is low, since use of co-trimoxazole is low and it is known that increased prevalence of resistance to co-trimoxazole is consistent with continuous use (Satoshi et al., 2002). All three studies were hospital based, therefore important to understand the contribution of the environment in antibiotic resistance because it may contribute to severe disease that may take longer to treat, longer hospitalization, failure of therapy and necessitating treatment with stronger more expensive antibiotics and increased likelihood of death (Ashbolt *et al.*, 2013).

Because the study area's aquifer is shallow, it is vulnerable to contamination from emerging pollutants such as antibiotics. Informal settlement dwellers are at a high risk of exposure because they live in crowded areas due to increased population, which leads to an increase in demand for services, causing more amplified contamination of water sources due to increased number sanitation facilities like pit latrines. These sanitation facilities are normally of poor quality and located near water points, allowing microorganisms to easily migrate from highly contaminated points to water points. In informal settlements, the spread of antibiotic resistance may be difficult to reduce due to the limiting factor that have been found to be sanitation, hygiene and disease transmission (Omulo *et al.*, 2021).

### **Occurrence of *Cryptosporidium parvum* in groundwater**

*Cryptosporidium parvum* is a microscopic parasite that cause cryptosporidiosis, a life threatening diarrheal disease, especially among the immunocompromised patients (Gharpure *et al.*, 2019; King *et al.*, 2019; Pignata *et al.*, 2019). There are a variety of species that affect animals as are zoonotic in nature and has a potential to cause outbreaks (Pignata *et al.*, 2019). The parasite consists of a protective outer shell (cyst) that make it possible to survive outside the host's body for long periods of time, thus are tolerant to conventional water treatment mechanisms like chlorination. *C. parvum* can be spread in different ways, but water i.e. drinking and recreational water are the most common ways to spread through the fecal-oral route (Gharpure *et al.*, 2019).

*Cryptosporidium* species is generally an important waterborne pathogen which is also a leading cause of morbidity and mortality from gastrointestinal infections that are zoonotic in nature and common among children, pregnancy and people with compromised immunity (Hassan *et al.*, 2021; Dupont *et al.*, 1995; Savioliet *et al.*, 2006). Detection can be a challenge due to the complex nature of the water matrix. Common methods of detection are visualization under a microscope using acid-fast staining as well as fluorescent staining after concentration (Kuzehkanan *et al.*, 2011).

However, detection using PCR has a higher sensitivity and specificity than microscopy and immunology techniques and has been used to detect *cryptosporidium* oocysts in environmental samples like water and in both human and animal fecal matter (Koehler *et al.*, 2017). This study utilized the QIA amp DNA stool mini kit (Qiagen, Hilden, Germany) to isolate and analyze for *C. parvum* which has been evaluated and proven to provide rapid tool for removing PCR inhibitors. The nested-PCR assay is sensitive, rapid and a more cost effective method for detection of *C. parvum* oocysts from water samples (Nikaeen & Makimura, 2007).

Groundwater sources are regarded pure and safe for drinking, due to the sub-surface protection, but the *cryptosporidium* status is unknown. The relative frequency of *Cryptosporidium* spp in global groundwater supplies are largely underreported, representing a gap in knowledge. Nonetheless, a review by Chique *et al* 2020 found that cryptosporidium is present in domestic groundwater sources, which presents a health concern about the users (Chique *et al.*, 2020). This study therefore sets pace for implementation of microbiological monitoring systems for groundwater to ascertain the transport of cryptosporidium to avert risk of infections.

*C. parvum* was detected in all water sources in the study site in informal settlements of Kisumu County, contrary to study conducted in Meru in surface water through various seasons where 8.3% (n=9) of all water samples were positive for *C. parvum*, which was attributed to precipitation (Ascolillo, Mutwiri, & Griffiths, 2009). Our study is consistent with a study in Italy which reported presence of both *C. parvum* and *C. hominis*, which according to literature have been found to contaminate surface water and treated water globally (Pignata *et al.*, 2019). A study in the USA Minnesota found 40% of 145 wells (groundwater) contaminated with *C. parvum*, 2 with *C. hominis* and 2 with *C. andersoni* (Stokdyk *et al.*, 2019). The contamination was linked to outbreaks between 2009 and 2017, with transmission occurring through recreational water, environmental contact, person-to-person spread, drinking water and food (Stokdyk *et al.*, 2019). A Colombian study investigated *C. parvum* was one of the species in raw and treated water, where the species were only detected in raw water in both rural and urban settings (Sánchez *et al.*, 2018). A study in Iran isolated *Cryptosporidium* sp from human and animal samples using the QIA amp DNA stool mini kit (Qiagen, Hilden, Germany).

A descriptive review of global prevalence, risk factors and knowledge gaps of *Cryptosporidium* species in groundwater supplies intended for human consumption over a period of 27 years reported detection of oocysts (Chique *et al.*, 2021). Majority of the studies were conducted in Europe and North America with only one study reported in Africa (Chique *et al.*, 2021).

*Cryptosporidium* has been shown to display seasonal peaks due to activities like agricultural, human waste production and local climate like rainfall (Lal *et al.*, 2019; Sterk *et al.*, 2016). The current climate change forecasts have predicted heavier and more persistent rainfall that is likely to result to higher aquifer recharge rates particularly in shallow water tables (Forzieri *et al.*, 2017) as is the case with the current study site.

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### **Assessment of human health risk of exposure to antibiotics in the environment in five informal settlements in Kisumu, Kenya.**

The role of the environment in development of antibiotic resistance has not been given attention (Stanton *et al.*, 2022) thus the human health risk is barely understood. Human health risk assessment is the process of estimating the nature and probability that an adverse health effect will occur resulting from exposure to certain health hazards like pollutants in the environment (Ashbolt *et al.*, 2013; United States Environmental Protection Agency, 2012). With the increasing risk of morbidity and mortality as a result of infection with antibiotic resistant pathogens, it is important to conduct human health risk assessments. The concentrations of Sulfamethoxazole ranged from  $12.7 \pm 1.1$  ng/L to  $258.2 \pm 27.4$  ng/L (Mean concentration = 57.26 ng/L;  $SD = 27.440$ ). The hazard quotient computed and was found to be 0 (zero), which is less than 1, therefore there is no carcinogenic risk of exposure of sulfamethoxazole in the environment. However, it doesn't mean that presence of sulfamethoxazole is not harmful since *E. coli* was found to be resistant to the antibiotics. This is more complex since measuring risk of exposure to antimicrobial resistance organisms are not available. Hazard quotient is the ratio of potential exposure to a substance and the level at which no adverse effects are expected. If the adverse effects are not likely to occur, the hazard quotient is usually equal to 1 (one). On the other hand, a hazard quotient of more than 1 (one) does not necessarily mean harm will occur, are simple statements of whether and/or the extent an exposure concentration exceeds the reference concentration (RfC).

Toxicity indicators like  $LC_{50}$ ,  $EC_{50}$  and mean inhibitory concentrations ( $IC_{50}$ ) have been reported in various studies evaluating effects of antibiotics on non-target organisms. Residues in the

environment are known to react and form complex mixtures, which implies that reactions that involve additivity, synergism or even antagonism are a possible scenario in the environment, thus increasing risk of a negative effect. The risk of environmental toxicity is higher by up to 50-200% when as a mixture, compare to the individual parent compound. For this reasons, environmental risk assessments are necessary and should include assessment of occurrence of mixture of compounds.

A review by (Koopaei & Abdollahi, 2017), on health risks associated with pharmaceutical products in wastewater found that most studies found the ratio of MEC (measured environmental concentration) to PNEC (predicted no effect concentration) of the pharmaceutical products was mostly low. These low ratios of the pharmaceutical compounds in the water were found not to pose a public health risk in drinking water. However, caution need to be taken since the level of pharmaceuticals that cause resistance among microbes is not known. Analysis of pharmaceuticals from hospital wastewater reported high levels of pharmaceuticals that included trimethoprim (Mendoza *et al.*, 2015) which is administered in combination with sulfamethoxazole. This was considered to pose a risk to the environment, especially taking into account dilution and degradation which can result to bioaccumulation (Mendoza *et al.*, 2015).

Some environmental scientists are of the opinion that the PNEC needs to be considered as a current safety indicator. However, the antibiotic selection pressure in the environment can accelerate the development and distribution resistant pathogens (Bengtsson-palme & Larsson, 2016). Consistent to Polianciuc *et al* 2020, this study also wishes to affirm that regarding this, no regulatory systems are in place to assess such risks and urge that the environmental risk assessments for human medicinal products includes possibility of emergence and spread of resistance in the environment after contamination with antibiotics (Polianciuc *et al.*, 2020). For instance, a limit of 100ng/L antibiotic discharge in the environment has been recommended by Le *et al*, to protect the ecosystem and mitigate against the possibility of resistance development. The authors cite importance of integrating clinically relevant bacteria and more species of environmentally relevant bacteria into the test systems of the risk assessments and examine the ability of spread of resistant microbes from point of contamination (Le *et al.*, 2017). This study reports presence of an antibiotic used in the prevention and treatment of infection, especially among patients whose immunity is compromised. The study being in a region of Kenya where HIV/AIDS prevalence are reported to be higher than the national figure, we reiterate the importance comprehensive clinically relevant microbes to avert risks associated with morbidity and mortality in this vulnerable populations.

An assessment of effects of anthropogenic activities focused on antibiotic resistant bacteria in the environment, where the human health concern was environmentally induced antibiotic resistance, which is feared may adversely affect health by affecting drug efficacy resulting increased morbidity and mortality, especially in low and middle income countries (Grundmann *et al.*, 2011). A review of emerging pollutants in water environment; occurrence, monitoring, fate and risk assessment noted that there were challenges in extrapolation of effects of a single emerging pollutant detected in the laboratory or even the effects following exposure to multiple emerging pollutants in aquatic organisms (Tang *et al.*, 2019). These challenges should be addressed through other studies to ensure measures to protect the environment, animals and humans are put in place.

## CONCLUSIONS AND RECOMMENDATIONS

This study discovered that there is little information on antibiotic use and disposal at the household level, particularly in informal settlements, where infectious diseases are more prevalent and use of antibiotics is likely to be more. Disposal practices comparable to those known to lead to environmental contamination, including groundwater were also identified in the study area. In this context, increased surveillance of antibiotics usage and disposal patterns is critical. Accurate policies that govern antibiotic use and discharge into the environment are required as antibiotic misuse threatens the balance of ecosystems and human health.

Antibiotic sulfamethoxazole is usually available in combination with trimethoprim, but only sulfamethoxazole was detected in groundwater. We recommend further research to analyse for presence of trimethoprim, while taking into account seasonality and soil characteristics of the study area.

Because of the problem of antibiotic resistance due to overuse and incorrect disposal, teaching on safe antibiotic prescription should be integrated into the medical training for all cadres. This will be useful in ensuring that the correct information is conveyed on usage and disposal. The benefits of using antibiotics must be emphasised at the same time taking into account the effects of antibiotics in the environment. Governments should guarantee that antibiotic stewardship guidelines are implemented and adhered to both locally and globally.

Sulfamethoxazole resistance was discovered in *E. coli* isolated from groundwater. The antibiotic is used to treat infections, particularly urinary tract infections, which are frequent in HIV/AIDS patients. The study recommends follow-up research on antimicrobial resistance detected in groundwater sources in the region to eliminate potential sources of resistance and avoid further spread.

*Cryptosporidium parvum* is a protozoan responsible for a significant portion of diarrhoea and severe morbidity among people with compromised immunity like HIV/AIDS patients. However, due to funding limits, antimicrobial sensitivity was not performed; consequently, more research is recommended.

The human health risk assessment indicates that there is no risk of exposure to the antibiotic sulfamethoxazole in groundwater. However, there presence of antibiotic resistant *E. coli* concerning since the sources of resistant bacteria have not been identified and there are difficulties in extrapolating the effects of a single emerging pollutant detected in the laboratory or even the effects following exposure to multiple emerging pollutants in aquatic organisms. Innovative assessments that take into account the obstacles should be designed avert the risk of exposure and its impact on health.

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## APPENDICES

### INFORMATION SHEET FOR THE HOUSEHOLDS



#### SCHOOL OF PATHOLOGY

#### INVESTIGATION OF CONTAMINATION OF COMMUNITY GROUNDWATER SOURCES WITH ANTIBIOTICS IN INFORMAL SETTLEMENTS IN KISUMU, KENYA

#### INTERVIEW – INFORMATION SHEET FOR HEADS OF HOUSEHOLDS

##### Introduction

Good morning/afternoon to you. My name is Kellen KARIMI, a PhD student from the Department of Clinical Microbiology in the University of the Witwatersrand, South Africa. I am required to conduct a research project (title above) as part of my degree studies. I am inviting you to consider participating in this study.

***Please kindly receive and read this information sheet and make an informed decision.***

This is your copy.

##### What is the study about?

This study is on groundwater contamination with antibiotics in informal settlements of Kisumu County. Antibiotics can be harmful, as they may lead to development of antibiotic resistance in certain circumstances, which is a public health concern. The purpose of this interview is to determine if the antibiotics being studied are present in water and to determine the presence of antibiotic resistance among microorganisms in the water. Persons volunteering to participate in the study will be asked questions pertaining to antibiotic use and disposal. Questions on groundwater consumption will be asked.

Water samples will be collected and analyzed for presence and levels of antibiotics and antibiotic resistance.

The study has been approved by the Kenyatta National Hospital/ University of Nairobi- Ethical Review Committee (KNH/UoN- ERC) (Protocol Number **P719/10/2018**).

### **Why me?**

You are being invited to participate in this study because you are the head of household, you reside in this Informal settlement and consume water from a shallow well.

To be able to understand antibiotic use and disposal, I think you are the most appropriate person. I would like to learn the practices on antibiotic use and how unused medication is disposed of, perhaps leading to contamination of groundwater in this region. Antibiotics may also find their way into groundwater in the excretions of a person taking them, especially in an area where there are no proper toilet facilities.

### **What will I be asked to do?**

I will ask you to spare some 20 minutes of your time for a questionnaire-based interview with me. This interview will be held here in your home at this time if you allow me. The responses to the questions from all study participants will be entered into a computer for analysis, to enable me get meaningful answers to my questions.

### **How do I benefit?**

The principal benefit of participating in this study is that you will have an opportunity to learn about form of contaminants that pollute groundwater, which you may be able to utilize to contribute to your general health and wellbeing and that of the persons living in your household.

If you agree to participate in this study, you may also benefit other people in this region and elsewhere, since the results will be used for educational purposes regarding the effects of misuse

and disposal of antibiotics. The researcher hopes to be able to use the results in aggregate to inform Government policy on better groundwater treatment for consumption.

### **What are the risks involved?**

This study aims to determine potential risk to health. There are no bodily harms associated with participating in this particular study.

If there are questions in that you are uncomfortable answering, please let me know so that we can proceed to the next question. Seek clarification at all times during the course of the interview.

In case of discomfort during the study, which is highly unlikely, kindly feel free to report to me.

### **How will my confidentiality be protected?**

Given that we you will be required to volunteer information towards this study, I will ensure that your identity is protected by not using information that will directly identify you like your name and address. Unique codes will be assigned to you to ensure you cannot be traced after the interview.

I will conduct the interview away from groups of people to avoid other people listening to the conversation.

All the data collected from interview will only be accessible to me (the Principal Investigator) and any other authorized person like my supervisor. The data will be protected with a password, only known to me. The questionnaires with information will be locked away in storage that is in line with rules by the University of the Witwatersrand.

### **How will I receive feedback?**

The results of this study will be made available to the authorities responsible for water quality in this area. Together with the authorities, I will arrange for dissemination of the results to the people in this study area through workshops. I also will write a book (Thesis) which will be available for reading in the library. The results will also be published in journals which are available online.

### **What if I don't want to participate?**

The decision on whether to participate in this study is entirely yours. If you do not want to participate, there will be no adverse consequences. Even if you choose to participate in the study, you are liberty to opt out anytime during the interview. If you feel like you will not continue, let me know so that I can end the interview.

### **Payment**

There is no payment for participation in this study, nor will it cost you anything except your time.

### **Where can I get further information?**

In case you have any concerns or would like to get more information about this study, please feel free to contact my supervisor on the address provided here.

**Name:** Kellen Joyce Karimi  
**Institution:** University of Nairobi  
 College of Health Sciences, School of Public Health  
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**Email:** [karimikellen@gmail.com](mailto:karimikellen@gmail.com); [2012904@students.wits.ac.za](mailto:2012904@students.wits.ac.za)  
**Telephone:** +254(0)721263316

**Name:** Dr. Aijaz Ahmad, PhD  
**Institution:** Department of Clinical Microbiology & Infectious Diseases  
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**Telephone:** +27(0)117171000

**How do I agree to participate?**

After reading this Information Sheet and asking questions, I believe you will be able to make an informed decision on whether to take part in this study. If you do wish to participate, I will kindly ask you to indicate so by signing on the form provided hereafter.

Thank you for reading this Information Sheet.

September 2019

## **QUESTIONNAIRE**

**Area code:**

**Interviewer code**\_\_\_\_\_ **Area code**\_\_\_\_\_

**Research title:** Investigation of Contamination of Community Groundwater Sources with Antibiotics in Informal Settlements in Kisumu, Kenya

### **Sociodemographic characteristics**

1. Age in complete years
2. Age category:

- ☐ 20-24
- ☐ 25-29
- ☐ 30-34
- ☐ 35-39
- ☐ 40-44
- ☐ 45-59
- ☐ 60-64
- ☐ 65-69
- ☐ 70-74
- ☐ 75-79
- ☐ 80 and  
above

3. Sex

☐ M

☐ F

4. Weight \_\_\_\_\_

5. Would you know your HIV status?

☐ Y

☐ N

6. What is your HIV status? \_\_\_\_\_

### Antibiotic use

7. Have you or any member of your household used antibiotics in the past 1 month? (*If no go to 12*)

☐ Y

☐ N

8. Were the antibiotics prescribed by to you by a doctor? Y/N

a. If no, how did you get the antibiotics?

☐ Advice from a friend

☐ Previous experience

☐ Online search for symptoms

☐ Other \_\_\_\_\_ (specify)

9. Antibiotic use among household members

| Person using antibiotic | Sex | Age | Disease being treated | Number of days antibiotic was prescribed | Number of days antibiotic was taken |
|-------------------------|-----|-----|-----------------------|------------------------------------------|-------------------------------------|
| Household head          |     |     |                       |                                          |                                     |
| Member 1                |     |     |                       |                                          |                                     |
| Member 2                |     |     |                       |                                          |                                     |
|                         |     |     |                       |                                          |                                     |
|                         |     |     |                       |                                          |                                     |

**Antibiotic disposal**

10. Do you always complete your antibiotic dose? Y/N

- ☐ Y  
☐ N

11. If no, where do you keep the remaining medicine?

- ☐ Keep for another time the illness occurs  
☐ Throw in the pit latrine  
☐ Throw in the compost pit  
☐ Burn  
☐ Not applicable

**Awareness of health effect of exposure to antibiotics in water**

12. Are you aware of health effects as a result of exposure to antibiotics in water?

- ☐ Y  
☐ N

13. Do you use water from the groundwater source near you?

- ☐ Y  
☐ N

a. If yes, what do you use the water for?

- ☐ Drinking  
☐ Cooking  
☐ Washing utensils  
☐ Washing clothes  
☐ Cleaning the house

14. How many people are in this household? \_\_\_\_\_

15. How long have you been using the groundwater source for the purposes mentioned?

- ☐ Drinking  
☐ Cooking  
☐ Washing utensils  
☐ Washing clothes  
☐ Cleaning the house

THANK YOU

## **KEY INFORMANT INTERVIEW INFORMATION SHEET**



### **SCHOOL OF PATHOLOGY**

### **INVESTIGATION OF CONTAMINATION OF COMMUNITY GROUNDWATER SOURCES WITH ANTIBIOTICS IN INFORMAL SETTLEMENTS IN KISUMU, KENYA**

#### **INTERVIEW – INFORMATION SHEET for KEY INFORMANTS**

#### **Introduction**

Good morning/afternoon to you. My name is Kellen KARIMI, a PhD student from the Department of Clinical Microbiology in the University of the Witwatersrand, South Africa. I am required to conduct a research project (title above) as part of my degree studies. I am inviting you to consider participating in this study as a Key Informant.

***Please kindly receive and read this information sheet and make an informed decision.***

This is your copy.

#### **What is the study about?**

This study is on groundwater contamination with antibiotics in informal settlements of Kisumu County. Antibiotics can be harmful, as they may lead to development of antibiotic resistance in certain circumstances, which is a public health concern. The purpose of this interview is to determine if the antibiotics being studied are present in water and to determine the presence of antibiotic resistance among microorganisms in the water. Persons volunteering to participate in the study will be asked questions pertaining to antibiotic use and disposal. Questions on groundwater consumption will be asked.

Water samples will be collected and analyzed for presence and levels of antibiotics and antibiotic resistance.

The study has been approved by the Kenyatta National Hospital/ University of Nairobi- Ethical Review Committee (KNH/UoN- ERC) (Protocol Number **P719/10/2018**).

### **Why me?**

You are being invited to participate in this study because you are the County Officer of Health in Kisumu County (the title changes depending on the officer being interviewed). You work in the County of Kisumu and are responsible for decisions on health matters affecting the people of Kisumu County.

To be able to understand the policy issues surrounding antibiotic use and disposal, as well as measures taken as a result, I think you are the most appropriate person. I would like to learn the measures put in place by the County to curb practices that can result to ill health in the population as a result of contamination of water/ environment with antibiotics as a result of practices on antibiotic use and how unused medication is disposed of by the population, perhaps leading to contamination of groundwater in this region. Antibiotics may also find their way into groundwater in the excretions of a person taking them, especially in an area where there are no proper toilet facilities.

### **What will I be asked to do?**

I will ask you to spare some 20 minutes of your time for an interview with me. During the interview, I will ask you questions which you will respond to the best of your knowledge and ability. I will request to record the audio to make it easier for me to write down the responses, analyze and compile a report about the subject. This interview will be held here in your office at this time if you allow me.

### **How do I benefit?**

The principal benefit of participating in this study is that you will have an opportunity to learn about form of contaminants that pollute groundwater, which you may be able to utilize to contribute to your general health and wellbeing and that of the persons living in your household.

If you agree to participate in this study, you may also benefit other people in this region and elsewhere, since the results will be used for educational purposes regarding the effects of misuse and disposal of antibiotics. The researcher hopes to be able to use the results in aggregate to inform Government policy on better groundwater treatment for consumption.

### **How will my confidentiality be protected?**

Given that we you will be required to volunteer information towards this study, I will ensure that your identity is protected by not using information that will directly identify you like your name and address. Throughout the recording, your name will not be mentioned to ensure anonymity.

All the data collected from interview will only be accessible to me (the Principal Investigator) and any other authorized person like my supervisor. The data will be protected with a password, only known to me. The questionnaires with information will be locked away in storage that is in line with rules by the University of the Witwatersrand.

### **How will I receive feedback?**

The results of this study will be made available to the authorities responsible for water quality in this area. Together with the authorities, I will arrange for dissemination of the results to the people in this study area through workshops. I also will write a book (Thesis) which will be available for reading in the library. The results will also be published in journals which are available online.

### **What if I don't want to participate?**

The decision on whether to participate in this study is entirely yours. If you do not want to participate, there will be no adverse consequences. Even if you choose to participate in the study, you are liberty to opt out anytime during the interview. If you feel like you will not continue, let me know so that I can end the interview.

### **Payment**

There is no payment for participation in this study, nor will it cost you anything except your time

### **Where can I get further information?**

In case you have any concerns or would like to get more information about this study, please feel free to contact my supervisor on the address provided here.

**Name:** Kellen Joyce Karimi

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### **How do I agree to participate?**

After reading this Information Sheet and asking questions, I believe you will be able to make an informed decision on whether to take part in this study. If you do wish to participate, I will kindly ask you to indicate so by signing on the form provided hereafter.

Thank you for reading this Information Sheet.

June 2019

## **KEY INFORMANT INTERVIEW GUIDE**

### **a. Department: Water and sanitation/ Health**

**Interview code** \_\_\_\_\_

**Research title:** Investigation of Contamination of Community Groundwater Sources with Antibiotics in Informal Settlements in Kisumu, Kenya

Designation of county official: \_\_\_\_\_

Highest level of education: \_\_\_\_\_

Knowledge of contamination of groundwater sources with emerging contaminants

Knowledge of contamination of groundwater sources with pharmaceutical compounds

Knowledge of contamination of groundwater sources with antibiotics

Measures taken to ensure water quality in Kisumu County

In your opinion, based on the new knowledge of contamination what should be done to improve water quality for populations

## PUBLICATIONS

## Publication 1



International Journal of  
*Environmental Research  
and Public Health*



## Article

# Prevalence of Antibiotic Use and Disposal at Household Level in Informal Settlements of Kisumu, Kenya

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**Abstract:** The use and abuse of antibiotics are directly related to the development of drug resistance, a global public health problem. Whereas the majority of research focus is on the use and misuse of antibiotics in drug resistance development, little is known about improper disposal, as a source of contamination in the environment that includes groundwater, especially in informal settlements. This study sought to determine antibiotic use and disposal in informal settlements in Kisumu, Kenya. A random cross-sectional sample of 447 households in selected informal settlements of Kisumu, Kenya was studied. A structured questionnaire was issued to persons heading households. The prevalence of antibiotic use was 43% (n = 193). Among these people, 74% (n = 144) had consulted a health worker in a healthcare facility for a prescription. Respondents did not always complete doses but kept the remainder for the next time they would become ill (54%). About 32% disposed of the remainder of the antibiotics in pit latrines and compost pits (10%) while 4% disposed through burning. Antibiotic use was fairly high despite a low level of awareness of the health effects of consuming water contaminated with antibiotics (35%) (n = 156);  $p = 0.03$ . Misuse and inappropriate disposal of antibiotics as identified may lead to a higher risk of antibiotic resistance, increasing the disease burden in the informal settlements.

**Keywords:** antibiotic use; antibiotic disposal; human health effect; antibiotic resistance; environment; informal settlements



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## 1. Introduction

Bacterial infections are a leading cause of morbidity and mortality around the world. Antibiotics continue to be a low-cost solution to current human and animal health problems both in treatment and prophylaxis [1,2]. They improve health outcomes, along with improved water and sanitation, nutrition, and vaccination [3]. The positive gains, however, are offset by rising antimicrobial resistance, which is facilitated by increased antibiotic consumption, with many low- and middle-income countries lacking the capacity to conduct surveillance [3]. According to the World Health Organization, approximately 50% of antibiotics consumed are unnecessary [4], resulting in antibiotic effectiveness being compromised owing to resistance [5–7]. Individual patients' antibiotic use is defined as when they receive the correct medicine for the appropriate indication, in doses that match their individual requirements for a specified period of time, and with the correct information [8]. Irrational antibiotic use, on the other hand, occurs when any of the predefined conditions are not met, or when the existing algorithm for determining the presence of infection among patients to warrant a prescription is not followed [6].

Antibiotic consumption increased by 39% globally between 2000 and 2015 [9]. Consumption rates were found to be high in North America, Europe, and the Middle East [3].

Consumption is lower in low- and middle-income countries, despite the fact that the bacterial disease burden that causes death is higher in low-income countries [10–12], but the LMICs are catching up and may soon register rates comparable to high-income countries [9]. Global consumption rates were reported to have increased by 46% overall [3].

Antibiotic use has been prevalent in hospital settings, where treatment guidelines recommend optimal antibiotic use in order to reduce the threat of antibiotic resistance, which worsens the burden of infectious diseases [12,13]. Antibiotic use has been reported in low- and middle-income countries, both in hospital settings [14] and in communities where misuse is common, with the majority preferring self-medication [15]. Misuse is largely fuelled by the ease of obtaining antibiotics without a prescription, a scenario described as less time-consuming, less expensive, and more convenient than visiting health facilities for a medical review [15]. In Kenya, an antibiotic consumption study was conducted in an informal settlement in Nairobi, where the prevalence of antibiotic use was found to be high (87% (initial survey) and 70% (follow-up survey)). This happened in addition to a lack of understanding about effective antibiotic use. [14].

The World Health Organization reported the prevalence of antibiotic consumption in defined daily dosages for four Sub-Saharan African countries: the Republic of Tanzania, Burkina Faso, Cote d'Ivoire, and Burundi, with defined daily dosages of 27.3, 13.8, 10.7, and 4.4 per 1000 inhabitants per day, respectively [16]. However, no data on antibiotic usage have been reported in Kenya, making it impossible to implement the set antibiotic use and antibiotic resistance guidelines [17].

Antibiotics are used for the treatment of infection and for prophylaxis among different populations [18], especially persons living with HIV patients, who use co-trimoxazole (sulfamethoxazole and trimethoprim) for prophylaxis and treatment of related infections [19]. For instance, among HIV patients, the World Health Organization recommends initiation of co-trimoxazole in patients with HIV clinical disease (stages 3 and 4) and/or a CD4+ cell count of  $\leq 350$  cells [19,20]. With the threat of antibiotic resistance, HIV patients are at risk of experiencing side effects that might lead to an increased burden of disease and death.

Antibiotic misuse has been categorized as both community- and healthcare-provider-driven owing to a lack of understanding and awareness of the dangers of antibiotic abuse in the community, availability of antibiotics without a prescription, and consumption of leftover medications, even though prescribers and dispensers were found to have adequate knowledge on antibiotic use [7,21]. The leftover antibiotics are available because patients are unable to complete the medication provided to them for a variety of reasons that vary from person to person, such as adverse effects, feeling better, or drug expiry [22,23]. Predictors of antibiotic misuse among healthcare providers include pharmacists' and prescribers' knowledge, attitude, and practice, patient/doctor contact, a lack of rapid diagnostic tests, pharmaceutical promotion, and a lack of adequate antibiotic education for healthcare professionals [7,21].

Environmental contamination with antibiotics has been linked to a number of sources, including pit latrines, sewage, wastewater, and animal and hospital waste disposal [24–28]. Other sources of groundwater contamination include industrial waste, agricultural runoff, and floods, which are distressing to the locals of these settlements studied and are linked to the shallow aquifer [29]. Antibiotics are becoming more and more prevalent in the environment, but it is unclear how they are used and disposed of in homes, given that they are present in water and what role they play in unintentional exposure [30]. To prevent environmental pollution, which fosters the development of antibiotic resistance, it is critical to properly and safely dispose of any unused drugs [18].

An increased urban population has resulted in the expansion of informal settlements, which has a negative influence on water supplies [31]. Residents of informal communities must consequently balance the scarcity with readily available groundwater by sinking shallow wells [32]. As the technology required to make water safe may not be readily available to informal settlement dwellers, groundwater may be consumed without being

subjected to any type of treatment owing to its clean appearance. Residents who are unaware of the presence of antibiotics in their drinking water may be at risk of exposure.

Since there exists a complex interaction between humans, animals, and the environment [33], health protection in a contaminated environment is critical. This strategy will attempt to attain optimal health while also addressing the future global antibiotic resistance burden, particularly in informal settlements [34]. This paper examines antibiotic use and disposal in informal settlement households.

## 2. Materials and Methods

The study was conducted in Kisumu County, which is located in Kenya's western area, 350 km from the capital city of Nairobi, at longitudes 33°20' E and latitudes 0°20' S and 0°50' S [35]. Kisumu has a total land area of 2086 km<sup>2</sup>, of which roughly 567 km<sup>2</sup> is water. The county is located on the beaches of Lake Victoria, the world's third-biggest freshwater lake, which also borders Kenya, Uganda, and Tanzania. The research sites are located in the Kisumu East and Kisumu Central sub-counties. There are eight informal settlements in the county, five of which were studied: Obunga, Manyatta A, Manyatta B, Nyalenda A, and Nyalenda B.

The population density of the informal settlements is projected to be 567,983 [36], with an annual urbanization rate of 2.8 percent [36]. The population in informal settlements relies on groundwater to supply their residential water needs while also using the sub-surface for sanitation facilities. The majority use pit latrines [32].

A cross-section of households in the chosen informal settlements was studied in September 2019. The AF-RIWATSAN project site, which included a number of components, was purposively selected [37]. Prior to the study, groundwater sources were mapped, and a sample of homes was selected at random, based on the population of each informal settlement around the identified groundwater sources [38]. A sample size of 442 households was determined. A proportionate distribution of homes was used, depending on the population of each of the five (5) informal settlements in the study area, namely Manyatta A and B, Nyalenda A and B, and Obunga, as reported by the Kenya Bureau of Statistics, to guarantee that each was sufficiently sampled. This ensured that more participants were drawn from more populous informal settlements, and vice versa. One individual from each home was chosen as the head to be involved in the study and interviewed after providing informed consent. The first household was chosen based on its proximity to the informal settlement's administrative leadership, being the one on the right-hand side of the chief's office. Every fifth family was chosen and included in the sample until the determined sample size [39] of 442 households was achieved.

A structured questionnaire was administered by trained interviewers using the Swahili language which was understood by the respondents. To verify that the translation from English to Swahili was not lost, the validity of the questions was maintained, and the possibility for introducing information bias was reduced, the questionnaire was piloted among residents of a different informal community within Kisumu. Groundwater use and the use of antibiotics were outcome variables. Sociodemographic traits, the source of the antibiotics, the completion of the antibiotic dose, and the disposal of leftover drugs were all predictor variables. Version 20 of IBM\* SPSS\* Statistics was used to enter the data, and also conduct descriptive analysis and cross tabulations whereas STATA 14 was used for logistic regression analysis. Continuous data were summarized using descriptive statistics, whilst associations and linkages in categorical variables were evaluated using chi-square tests. To find predictors of antibiotic use, logistic regression analysis was utilized.

Ethical clearance to conduct this research was obtained from the Health Research Ethics Committee of the University of the Witwatersrand, Johannesburg South Africa (HREC. Protocol Number M190412); the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UoN-ERC. Ref. No. P71910/2018); and the National Commission for Science and Technology and Innovation. Ref No. NACOSTI/P/19/3232/28732). To protect privacy, study areas were coded.

### 3. Results

#### 3.1. Participant Information

In the five informal communities, 447 households were investigated. Of these, 75% ( $n = 337$ ) of responders were female, whereas 25% ( $n = 110$ ) were male. Table 1 shows the sociodemographic characteristics of the participants, including their gender, age, place of residence, and HIV status. The majority of the female responders were under the age of 45 (79%). The respondents' minimum age was 24 and their maximum age was 80. Manyatta A had the most participants (32.1%), followed by Nyalenda A (21.4%), Nyalenda B (21.2%), Manyatta B (19.9%), and Obunga (5.4%). As HIV affects the immune system, making people more susceptible to infections and, as a result, to increased antibiotic use, we needed to know the HIV status of the respondents; only around 5% of the participants were HIV positive.

**Table 1.** Sociodemographic and clinical characteristics of the study participants.

| Characteristic | Description | n (%)       |
|----------------|-------------|-------------|
| Sex            | Male        | 110 (25.0%) |
|                | Female      | 375 (75.0%) |
| Age            | 20–24       | 65 (14.5%)  |
|                | 25–34       | 173 (38.6%) |
|                | 35–44       | 115 (25.7%) |
|                | 45+         | 94 (21.2%)  |
|                | OBUNGA      | 24(5.4%)    |
| Settlement     | MANYATTA A  | 143 (32.1%) |
|                | MANYATTA B  | 89 (19.9%)  |
|                | NYALENDA A  | 96 (21.4%)  |
|                | NYALENDA B  | 95 (21.2%)  |
| HIV status     | Positive    | 21(4.7%)    |
|                | Negative    | 398 (88.8%) |

#### 3.2. Prevalence of Antibiotic Use

Antibiotic use in the selected households during the previous month from the day of the interview was reported to be 43%. (193). Among individuals who consumed antibiotics, 70% ( $n = 137$ ) reported getting the antibiotic through a prescription, whereas the rest did not consult a doctor for the antibiotic prescription. Further examination in the households that reported self-medication revealed that antibiotics were either used based on previous experience (76%) or on the advice of friends (26%).

Additionally, the level of awareness of the health implications of drinking antibiotic-contaminated water was evaluated and it was discovered that only 35% ( $n = 158$ ;  $p = 0.003$ ) of households who reported using antibiotics were aware of the health risks of drinking antibiotic-contaminated water. Table 2 summarizes an in-depth examination of the distribution of antibiotic sources based on the characteristics of the respondents. Remarkably, a large proportion (87.5%) of HIV + respondents received a prescription from a medical practitioner for antibiotics taken. In terms of different settlement areas, a relatively small percentage of respondents in Manyatta B obtained an antibiotic prescription from a medical practitioner, which could explain the substantial difference seen ( $p < 0.000$ ) among informal settlements.

Furthermore, to comprehend the amount of health consciousness The odds of employing antibiotics were calculated using a 95% confidence interval, and no statistically significant difference was observed (Table 3). Female individuals had higher odds of using antibiotics than male participants, with an odds ratio of 1.33;  $z = 1.24$ ,  $SE = 0.3105$ ,  $p > 0.05$ . Interestingly, the odds of using antibiotics were shown to be lower among HIV-positive participants than among HIV-negative participants (0.313;  $z = -1.73$ ,  $SE = 0.2097$ ,  $p > 0.05$ ). Participants aged 45 years and older showed a higher likelihood of using antibiotics compared to younger participants (odds ratio = 1.54,  $z = 1.22$ ,  $SE = 0.550$ ,

$p > 0.05$ ). In terms of geographical location, the situation differs among different settlements, with the use of antibiotics decreasing in only one informal settlement (Nyalenda B) (odds ratio =0.42).  $z = 1.07$ , SEM = 1.726156, the observations were judged to be within one standard deviation of the mean.

**Table 2.** Percentage distribution of sources of antibiotics used against demographic and clinical characteristics of the study participants.

| Characteristics |            | Source of Antibiotic Used    |                           |                           |                     |                       | p-Value |
|-----------------|------------|------------------------------|---------------------------|---------------------------|---------------------|-----------------------|---------|
|                 |            | Prescribed by a Doctor % (n) | Advice from Friends % (n) | Previous Experience % (n) | Online Search % (n) | Other (Chemist) % (n) |         |
| Sex             | Male       | 77.1 (37)                    | 40.0 (4)                  | 80.0(8)                   | 0.0(0)              | 1.7(2)                | 0.408   |
|                 | Female     | 73.8(107)                    | 24.3(9)                   | 75.7(28)                  | 2.7(1)              | 1.2(4)                |         |
| HIV status      | Positive   | 87.5 (14)                    | 0 (0)                     | 100 (2)                   | 0(0)                | 0 (0)                 | 0.001   |
|                 | Negative   | 75.2(121)                    | 31.6(12)                  | 76.3(29)                  | 2.6(1)              | 1.3 (5)               |         |
| Settlements     | OBUNGA     | 81.8(9)                      | 0 (0)                     | 50 (1)                    | 0 (0)               | 4.2 (1)               | 0.000   |
|                 | MANYATTA A | 81.6(62)                     | 0 (0)                     | 100 (13)                  | 0 (0)               | 0 (0)                 |         |
|                 | MANYATTA B | 64.9 (24)                    | 69.(9)                    | 84.6(11)                  | 0 (0)               | 1.1 (1)               |         |
|                 | NYALENDA A | 70.2 (33)                    | 28.6 (4)                  | 71.4 (10)                 | 7.1 (1)             | 0 (0)                 |         |
|                 | NYALENDA B | 72.7 (16)                    | 0 (0)                     | 20.0(1)                   | 0 (0)               | 4.2 (4)               |         |
|                 | 20–24      | 70.8 (17)                    | 71.4 (5)                  | 28.6 (2)                  | 0 (0)               | 4.6 (3)               |         |
|                 | 25–34      | 72.7 (48)                    | 77.8 (14)                 | 83.3 (15)                 | 5.6 (1)             | 1.2 (2)               |         |
| Age             | 35–44      | 73.6 (39)                    | 69.2 (9)                  | 100 (13)                  | 0 (0)               | 0 (0)                 | 0.086   |
|                 | 45+        | 80.0 (40)                    | 66.7 (6)                  | 66.7 (6)                  | 0 (0)               | 1.1 (1)               |         |

**Table 3.** Predictors of antibiotic use.

| Antibiotic Use |          | Odds Ratio | Std. Error | z     | p > z | 95% Conf. Interval |
|----------------|----------|------------|------------|-------|-------|--------------------|
| Sex            | Female   | 1.33       | 0.3105     | 1.24  | 0.215 | 0.845–2.105        |
|                | Positive | 0.313      | 0.2097     | −1.73 | 0.083 | 0.084–1.163        |
| Age (years)    | 25–34    | 0.96       | 0.302      | −0.12 | 0.908 | 0.522–1.782        |
|                | 35–44    | 0.99       | 0.335      | −0.03 | 0.980 | 0.511–1.921        |
|                | 45+      | 1.54       | 0.550      | 1.22  | 0.223 | 0.768–3.104        |
| Settlements    | MN A     | 1.54       | 0.721      | 0.91  | 0.362 | 0.611–3.855        |
|                | MN B     | 1.17       | 0.571      | 0.32  | 0.748 | 0.449–3.042        |
|                | NY A     | 1.61       | 0.778      | 0.98  | 0.325 | 0.623–4.153        |
|                | NY B     | 0.42       | 0.207      | −1.76 | 0.078 | 0.157–1.103        |
| Cons           |          | 2.263088   | 1.726156   | 1.07  | 0.284 | 0.507–10.091       |

OBG—Obunga; MN A—Manyatta A; MN B—Manyatta B; NY A—Nyalenda A; NY B—Nyalenda B.

### 3.3. Antibiotic Disposal

Proper disposal of leftover antibiotics is regarded as a major concern and has been linked to the development of antibiotic resistance. In this study, all participants were interviewed about the various methods they disposed of leftover medicines. Respondents disposed of antibiotics in a variety of methods, including 51.6% ( $n = 16$ ) in pit latrines, 16.1% ( $n = 5$ ) in compost pits, and 6.5% ( $n = 2$ ) by burning. However, the majority of respondents (87.1%;  $n = 27$ ) did not discard the unfinished antibiotics but preserved them for future use (Table 4). Antibiotic completion analysis revealed that females completed their antibiotic doses more than males.

A statistically significant difference in HIV status was observed, ( $p = 0.000$ ), with the majority of HIV-positive respondents completing their antibiotic dose and just a tiny fraction of HIV-negative respondents not completing their antibiotic dose. There was also a difference among the informal settlements ( $p = 0.001$ ), with a lower proportion of responders in Nyalenda B completing their antibiotic doses than in the other settlements. In terms of age group variations, it was discovered that antibiotic completion rose with age, although there were no significant differences in antibiotic dose completion across the various age groups (Table 4).

**Table 4.** Distribution of completion of antibiotic dose by demographic characteristics.

| Characteristics | Description | Yes        | No        | p-Value |
|-----------------|-------------|------------|-----------|---------|
| Sex             | Male        | 39 (32.5)  | 7 (5.8)   | 0.647   |
|                 | Female      | 117 (36.3) | 27 (8.4)  |         |
| HIV status      | Positive    | 15 (71.4)  | 1 (4.8)   | 0.000   |
|                 | Negative    | 130 (32.7) | 26 (6.5)  |         |
| Settlements     | MN A        | 63 (43.8)  | 13 (9.0)  | 0.001   |
|                 | MN B        | 30 (33.7)  | 6 (6.7)   |         |
|                 | NY A        | 35 (36.5)  | 11 (11.5) |         |
|                 | NY B        | 17 (17.9)  | 4 (4.2)   |         |
|                 | OBG         | 11 (45.8)  | 0 (0)     |         |
| Age             | 20–24       | 21 (32.3)  | 2 (3.1)   | 0.089   |
|                 | 25–34       | 55 (31.8)  | 10 (5.8)  |         |
|                 | 35–44       | 40 (34.8)  | 11 (9.6)  |         |
|                 | 45+         | 40 (42.6)  | 11 (11.7) |         |

OBG—Obunga; MN A—Manyatta A; MN B—Manyatta B; NY A—Nyalenda A; NY B—Nyalenda B.

### 3.4. Ground Water Use

Since improper disposal of unfinished antibiotics contaminates groundwater, it was critical to examine groundwater use in the selected settlements. As expected, more than 99% of respondents reported using groundwater for various household reasons (Table 5). While the majority of groundwater was reported to be used for washing clothes (96%), cleaning houses (95%), and washing utensils (78%), the same water was also seen to be utilized for drinking (9%) and cooking (18%) purposes. In terms of gender, a greater proportion of male respondents (12.6%) reported drinking groundwater than the opposite sex. HIV-positive subjects (19%) reported drinking from groundwater, but HIV-negative participants (8.9%) did not. Residents of the Manyatta B and Nyalenda B informal settlements were more likely to use groundwater for drinking than residents of the other settlements. We discovered that younger people did not drink groundwater, but that drinking groundwater increased with age. As water use is ubiquitous, no significant difference was found when comparing participant characteristics.

**Table 5.** Percentage distribution of groundwater use against sociodemographic characteristics.

| Characteristics |          | Groundwater Use |               |                        |                       |                     | p-Value |
|-----------------|----------|-----------------|---------------|------------------------|-----------------------|---------------------|---------|
|                 |          | Drinking n (%)  | Cooking n (%) | Washing Utensils n (%) | Washing Clothes n (%) | House Cleaningn (%) |         |
| Sex             | Male     | 12.6 (15)       | 21.8 (26)     | 82.2 (97)              | 94.9 (112)            | 94.9 (112)          | 0.261   |
|                 | Female   | 7.5 (24)        | 17.5 (56)     | 79.1 (253)             | 96.6 (309)            | 95.3 (303)          |         |
| HIV status      | Positive | 19.0 (4)        | 28.6 (6)      | 85.7 (18)              | 100.0 (21)            | 95.2 (20)           | 0.950   |
|                 | Negative | 8.9 (35)        | 18.0 (71)     | 79.2 (312) (79.2)      | 95.9 (378)            | 95.9 (376)          |         |
| Settlements     | OBG      | 12.5 (3)        | 16.7 (4)      | 62.5 (15)              | 95.8 (23)             | 95.8 (23)           | 0.716   |
|                 | MN A     | 1.4 (2)         | 9.9 (14)      | 92.2 (130)             | 100.0 (141)           | 92.8 (129)          |         |
|                 | MN B     | 13.5 (12)       | 25.8 (23)     | 74.2 (66)              | 93.3 (83)             | 95.5 (85)           |         |
|                 | NY A     | 10.5 (10)       | 20.0 (19)     | 80.0 (76)              | 97.9 (93)             | 95.8 (91)           |         |
|                 | NY B     | 13.7 (13)       | 24.2 (23)     | 69.5 (66)              | 91.6 (87)             | 97.9 (93)           |         |
| Age             | 20–24    | 3.1 (2)         | 12.3 (8)      | 72.3 (47)              | 95.4 (62)             | 90.8 (59)           | 0.660   |
|                 | 25–34    | 7.1 (12)        | 14.7 (25)     | 71.2 (121)             | 94.7 (161)            | 95.9 (163)          |         |
|                 | 35–44    | 9.6 (11)        | 23.5 (27)     | 89.6 (103)             | 99.1 (114)            | 94.7 (107)          |         |
|                 | 45+      | 16.0 (15)       | 24.5 (23)     | 87.1 (81)              | 95.7 (89)             | 97.8 (91)           |         |

OBG—Obunga; MN A—Manyatta A; MN B—Manyatta B; NY A—Nyalenda A; NY B—Nyalenda B.

#### 4. Discussion

Lack of accurate diagnosis, combined with self-medication, is a rising global problem that leads to antibiotic abuse, particularly in low- and middle-income countries. Antibiotic use was reported to be 43% among Kisumu County residents living in informal settlements. However, an entry and exit survey on antibiotic use in an urban informal settlement discovered a high prevalence of antibiotic use at 87% (entry) and 70% (exit) [14]. Antibiotic use in these informal settlements is higher than the level reported in Europe in 2016, where 34% were reported to have used antibiotics [40]. In this study, the majority (70%) of antibiotic users in informal settlements obtained the medication on a prescription, but some also obtained the medication after seeking advice from friends for advice. Other participants utilized leftover antibiotics from a previous experience and some obtained the antibiotics after doing an online search for the symptoms and going directly to a chemist. This is comparable to a comprehensive investigation conducted in Uganda, where it was revealed that self-medication is common in low-income countries, with an overall prevalence of 38.8% for different ailments [41]. Self-medication was viewed as more convenient, less expensive, and less time-consuming than getting a prescription from a medical facility [15]. Antibiotics were saved for future use from a variety of sources, including pharmacies, leftover doses, and drug sharing, with education, age, gender, previous successful usage, the severity of illness, and income identified as factors influencing the behavior [15,41]. In another study, despite legal requirements that antibiotics be dispensed only with a prescription, antibiotics were obtained without a prescription, owing to increased access and poor enforcement of the legislation [15]. Antibiotic overuse is exacerbated by the ease with which antibiotics can be obtained without a prescription; however, there is a scarcity of information on antibiotic use and/or misuse in Kenya [17]. This study did not determine whether the correct dosage was administered in order to determine dosage compliance, which is an important factor that should be investigated further.

Self-medication with antibiotics is a major contributor to the development of drug resistance [41] and is typically conducted for the user's convenience, resulting in antibiotic misuse [35]. Self-medication was found to be common in southern and eastern European countries with high levels of antibiotic resistance [42]. It has also been observed that the availability of leftover antibiotics is a key contributor to the habit of self-medication [43]. There is a need to develop a rigorous data collection system for antibiotic usage as well as to raise awareness about the health implications of antibiotic exposure in the environment, including groundwater.

An estimated 60% of Kisumu inhabitants live in informal settlements, where they face deficiencies such as a lack of clean water and basic sanitation [38]. Groundwater is utilized for household reasons by 99.8% of the people in these settlements, including cooking, drinking, washing utensils, washing clothes, and cleaning the house. This study supports the findings of a review of groundwater used for drinking in south-east Asia and the Pacific, which found that the average proportion of families using groundwater for drinking is high (66% (range of 22–95%) in urban areas and 60% (range of 17–93%) in rural regions) [44]. Okotto et al. (2015) discovered that an estimated 472m<sup>3</sup> of groundwater was extracted but was used for other domestic purposes than drinking and cooking in the Kisumu informal settlements [45]. Another study in Kiambu County, Kenya, investigated the extent of groundwater use for domestic and irrigation purposes and discovered that residents perceived groundwater to be contaminated, with only 36.7 percent of households using groundwater for domestic purposes and 13.3% using it for irrigation [46].

The improper disposal of unused, stored, and expired antibiotics is of major concern [47], as users may be unaware of suitable disposal measures [48]. Antibiotics contaminate groundwater in a variety of ways, including direct dumping with rubbish, faeces and urine excretion, and hospital and industrial waste [1,28,49,50]. The same pattern was found in this study, where respondents reported disposing of antibiotics in pit latrines, compost pits, or burning. Pit latrines have been reported to be the most commonly used form of sanitation in facilities in informal communities [51]. However, other causes of water con-

tamination have been identified, including industrial, agricultural, and flooding [46], which create calamity when it rains owing to the study area's shallow water table [29]. However, several respondents did not discard the antibiotic but saved it for future use. Antibiotic usage has been linked to an increase in the likelihood of incorrect disposal into the environment [52]. Keeping the remainder of antibiotics for use the next time a person becomes ill is risky since there is a chance that the antibiotics will expire. The efficacy and potency of the antibiotics are compromised and are bound to fail to treat the intended infection [53]. They are also harmful and likely to lead to toxicity that may lead to irreversible damage to body organs [54]. Unfortunately, antibiotic resistance caused by poor antibiotic disposal is hardly addressed and is not a priority even among healthcare practitioners [52]. The literature has demonstrated that contamination of groundwater sources is higher in places with a high population density [55], which is a typical feature in informal settlements, but information on antibiotic usage and disposal in informal settlements has not been documented.

People with weakened immune systems use antibiotics the most to combat opportunistic infections. In this study, the self-reported HIV status was 4.7%, with a higher likelihood of utilizing antibiotics and completing their antibiotic dosage. This study's location is in a region in Kenya where the HIV prevalence is greater than the national prevalence, which has been reported to be between 16.3% [56] and 17.5% [57]. An estimated 60% of Kisumu inhabitants live in informal settlements, where they face deficiencies such as a lack of access to services such as clean water and good sanitation [38]. The environment harbours resources such as groundwater, which serves as a complement to intermittent water supply for residential use, and it is critical that initiatives aimed at educating people living in informal settlements about the dangers of consuming unclean water are implemented [58]. As a result of knowing the HIV burden in the study region, knowing it follows that protecting the environment from antibiotic contamination is a key step in promoting human well-being in the one health setting. This study describes findings from a Kenyan informal settlement; however, it is well known that informal communities around the world face similar difficulties of access to essential amenities. The findings indicate that countries have a responsibility to carry out the global antimicrobial resistance action plan.

## 5. Implications for Policy and Areas for Further Research

Despite attempts to raise awareness about antibiotic usage and resistance through worldwide annual events, consumers still lack reliable knowledge about proper antibiotic use. To reduce misuse and abuse, it is critically important to design methods that assure measurement of antibiotic consumption by countries and use thereof at individual and household levels.

More research on prescribers' and users' knowledge of the amount of use and health impacts as a result of antibiotic exposure in the environment owing to inadequate disposal, should be conducted. It is also necessary to devote time to improving understanding through surveillance and study. These measures will reduce the disease burden and promote well-being, particularly in informal settlements, which have similar cross-cutting inequities.

Antibiotic disposal practices in informal communities have been found to pollute the environment, including groundwater; the result being environmentally induced antibiotic resistance. Antibiotic use reduction and correct disposal are strategies for combating antibiotic resistance in groundwater. Governments, as well as other relevant organizations and stakeholders, should prioritize the provision of safe water for all, particularly disadvantaged residents of informal settlements. Proper disposal techniques must be prioritized, and hence raising awareness about these among prescribers, providers, and antibiotic consumers should be a top concern.

### Limitations

Since this was an academic study with restricted resources, a large sample could not be studied. However, the study's findings are applicable to and can be generalized to

similar settings, because informal settlements have traits that are not necessarily unique to a particular geographical area. To look for differences between informal settlements, a different study approach than descriptive is recommended. For reason that the study was dependent on the response given, the actual HIV status of the respondents may not have been captured. A study targeting HIV patients at the clinic could be a better measure of the burden of disease among the informal settlements, and thus more concrete conclusions could be made on antibiotic use among HIV patients.

## 6. Conclusions

In Kenya, there is little information on antibiotic use at the household level. This study discovered that antibiotic use is significantly high, with disposal procedures similar to those known to contribute to environmental contamination, including groundwater. There was antibiotic misuse and abuse, as well as improper disposal, along with a lack of awareness of the health risks of antibiotic exposure in the environment, such as drinking antibiotic-contaminated water. The majority of informal settlement dwellers rely on groundwater to fulfill their water demands. As a result, there is a need for studies to increase surveillance of antibiotic usage and disposal. Additionally, governments and other relevant organizations must strengthen their commitment to groundwater resource management in order to offer sustainable universal water distribution free from the inclusion of pollutants such as antibiotics, particularly among disadvantaged communities in informal settlements.

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**Data Availability Statement:** Data are available on request due to the privacy of the respondents. The data presented in this study are available on request from the corresponding author. Data are not publicly available due since this is an academic study and some of the data are still being analyzed for publication.

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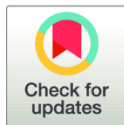
## RESEARCH ARTICLE

# Contamination of groundwater with sulfamethoxazole and antibiotic resistant *Escherichia coli* in informal settlements in Kisumu, Kenya

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## Abstract

High frequency of antibiotic detection in groundwater in informal settlements is attributed to increased usage and improper disposal, thus difficult to identify sources of antibiotic resistance in the environment, worsened by inadequate sanitation facilities and increased population density, particularly in developing-countries. Reliance on groundwater exposes them to pollutants and risk of antibiotic resistance, in addition to experiencing inequities in accessing vital services. Sulfamethoxazole and trimethoprim, used for prophylaxis by HIV/AIDS patients were tested in 49 groundwater sources in Kisumu, Kenya. Only Sulfamethoxazole (SMX) was found, with a detection frequency of 14.3% and concentrations ranging from below limit of quantification (LOQ) to 258.2 ng/L. Trimethoprim (TMP), marketed in combination with sulfamethoxazole, was not detected, owing to its high distribution coefficient (kPa7.12) and, generally, being a bigger molecule with modest water mobility and solubility. Furthermore, TMP ratio in cotrimoxazole is low (5:1), it is expected that mass loading will be lower, as well as influence of the study area's hydrogeology, where soil is clayey with high porosity and permeability. *Escherichia coli* was recovered in 98% (n = 48) of water samples, with counts ranging from 16 to 8,850 MPN/100ml. Additionally, resistance to sulfamethoxazole was identified in 6% (n = 3) samples with Inhibition Zone Diameters of 0.8mm(resistant), 10.5mm (resistant), and 11.5mm (intermediate), but not among samples where SMX was detected. Antibiotic concentrations in water that can cause resistance are unknown because antibiotic-resistant *E. coli* was not found in water samples where sulfamethoxazole was identified, raising concerns about environmental resistance spread. Concentration of SMX was lower in a previous research, which only collected water from one groundwater source, than the current study, which included additional samples (49). Presence of SMX and *Escherichia coli* resistance is of concern and necessitates greater attention and regular

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monitoring for potential contaminants and resistance trigger to avert potential risks to human health.

## 1. Introduction

Proliferation of urban informal settlements is as a result of increased urbanization, which has an impact on the limited resources available like improved water and sanitation facilities [1]. This happens in Low and Medium income Countries, and at least 50% of the urban population reside in those types of settlements [2]. The settlements have seen increased levels of inequality and exclusion, and lack of access to opportunities, employment, health, education, technology, municipal services and private goods [2, 3], a recipe for social unrest. To compensate for water scarcity, informal settlement dwellers utilize the readily available groundwater through construction of boreholes and shallow wells [4], without necessarily subjecting the water any form of treatment.

Higher prevalence of infectious diseases in informal settlements is a major driver for increased antibiotic use, leading to increased demand for antibiotics due to low capacity to access information to make informed health choices particularly in developing nations [5]. Antibiotic use in the informal settlement was found to be 43%, with the majority acquiring antibiotics without a prescription for a qualified health practitioner, choosing to self-medicate, which may accelerate selection and spread of antibiotic resistant microbes [6]. Access to antibiotics in informal settlements without a prescription is easy, where the health practitioners are not necessarily qualified or are not competent [7]. The unqualified practitioners are likely not to recognize infections that require further medical attention, thus resort to dispense different types of antibiotics at a more expensive cost. In such cases, residents whose purchasing power is limited end up either buying part of the dose, that may also be counterfeit or low quality [7]. As a result, the cycle of antibiotic self-medication leads to poverty and also antimicrobial resistance in general.

The efficacy of any antibiotics used to prevent and treat bacterial infection is critical in current human health practice [8]. However, the rise in antibiotic use particularly in the informal settlements is a reason to worry about due to the way the antibiotics are disposed bearing in mind possible negative impact on the environment, particularly on human health, due to the unknown influence of active pharmacological compounds in the environment from numerous sources. Antibiotics detected in the environment are largely attributed to improper use and disposal practices, coupled with incomplete metabolism of the pharmaceuticals in the human body, resulting in their excretion in urine and feces either unchanged or as active derivatives [9]. Antibiotics have been discovered to pollute groundwater sources via leaching from sanitation facilities such as pit latrines, waste disposal facilities in the subsurface in places where the aquifer is shallow, and sensitive to rainfall recharge, all of which impair the region's sanitation system [4]. Even at sub-optimal levels, cumulative continuous exposure to Active Pharmaceutical Ingredients (APIs) to non-target organisms may have detrimental eco-toxicological effects.

It is also known that current antibiotic removal technologies are not economically viable [10], and for many countries around the world, is yet to be made a regulatory requirement, so this water is not necessarily subjected to any form of treatment before consumption, exposing residents to the novel risk of antibiotics and their derivatives in drinking water, and selection for resistance in the environment. Health protection in a polluted environment is critical because there is a complex connection between humans, animals, and the environment [11]

interface that necessitates a one-health, multi-sector collaboration to obtain better public health outcomes.

The presence of antibiotics sulfamethoxazole (SMX) and trimethoprim (TMP) was investigated, as well as an antibiotic sensitivity test against *Escherichia coli* in groundwater sources. The World Health Organization recommends antibiotics for the prevention and treatment of infections in people living with HIV/AIDS [12]. Most infections, such as urinary tract infections (UTI) caused by enteric pathogen *Escherichia coli* and pneumocystis pneumonia caused by *Pneumocystis jirovecii*, can progress from asymptomatic to severe disease and death due to the increased susceptibility to disease in people living with HIV/AIDS [13]. This study location is reported to have a high HIV prevalence of 16.3% [14] (NACC, 2018) and the population is at risk of being exposed to antibiotic resistant *Escherichia coli*. It is also Sulfamethoxazole and trimethoprim are found together in a fixed dose of Cotrimoxazole, where each tablet includes 80mg Trimethoprim BP and 400mg Sulfamethoxazole BP, resulting in a 5:1 ratio [12]. *Escherichia coli* was also isolated from the water and antibiotic resistance determined.

*Escherichia coli* are generally harmless organisms that are members of the gut flora in both humans and warm blooded animals. They are also known to be an opportunistic pathogen and have been linked to intestinal illness, urinary tract infections, newborn meningitis, and gastroenteritis [15–17]. Contamination of water supplies by coliforms like *Escherichia coli* mostly is as a result of lack of adequate sanitation, which is a common phenomenon in informal settlements [18, 19]. The bacteria are reported as an indicator of fecal contamination in the assessment of water quality deterioration. There is however rising worry about the prevalence of antibiotic-resistant bacteria and antibiotic-resistant genes in aquatic environments, with industries, agriculture, and domestic antibiotic usage have all been identified as sources of aquatic pollution [20, 21]. Studies focusing on antibiotic resistance have concentrated in aquatic environments heavily contaminated with antibiotics as a result of activities like wastewater treatment and anthropogenic activities such as farming [22, 23]. Presence and spread of antibiotic resistance in natural water used for irrigation, aquaculture, recreation, drinking and other domestic purposes are ignored whereas the importance to human life is well known. The direct or indirect use of the water contaminated by antibiotic resistant bacteria could have an effect on the health of human beings, animals and contaminate the environment [24, 25].

The goal of assessing the extent of contamination in chosen informal settlements and antibiotic resistance is to enable for the evaluation of preventative measures for harmful health consequences especially among the HIV/AIDS patients whose immunities are compromised. This not only is of benefit to humans but also on animals and the general environment.

## 2. Materials and methods

### 2.1 Ethics statement

Ethical approval to conduct this research was obtained from the Health Research Ethic Committee of the University of the Witwatersrand (HREC. Protocol Number M190412); the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UoN-ERC. Ref No. P71910/2018). Research permit was provided by the National Commission for Science, Technology and Innovation (Ref No. NACOSTI/P/19/3232/28732). Permission to draw water samples was sought from the owners of the water points at household level. To guarantee that the research participants were not identifiable, confidentiality and anonymity were strictly maintained at all times.

**2.1.1 Description of study area and sample collection.** This was a descriptive cross-sectional study of households in Kisumu County's informal settlements with a groundwater supply. Prior to data collection, the groundwater sources in the study region were identified, and a

sample of 49 sources were chosen in proportion to the population in each informal community. Informal settlements, are located in Western Kenya, 350 kilometers from Nairobi, and are located between longitudes 33° 20'E and latitudes 0° 20'S and 0° 50'S [26]. Kisumu County covers an area of 2,086 km<sup>2</sup> and administers 567 km<sup>2</sup> of Lake Victoria waters, the world's third largest fresh water lake. There are eight informal settlements in the County, of which five have been researched. The study sites were selected purposively based on the initial larger study site under the AFRIWATSAN project partly located in the Kisumu East and Kisumu Central sub-counties (Fig 2).

The population density of the informal settlements is around 567,983 [27], with an annual urbanization rate of 2.8 percent [28]. The population in informal settlements relies on groundwater to supplement their domestic water needs while also using the subsurface for sanitation, primarily through the usage of pit latrines [4, 19, 29–31].

Water samples were collected in triplicate in 500 ml from 49 groundwater sources between February 9th and 15th, 2021, during this period the study area experienced light rains. The water was transported to the Kenya Bureau of Standards (KEBS) laboratory in Kisumu, where one (1) portion of it was set aside for isolation of *Escherichia coli* and antibiotic resistance testing. The other two (2) portions were stored at -4°C until they could be shipped to the KEBS Laboratory in Nairobi for analysis. Fig 1 shows the distribution of mapped groundwater points.

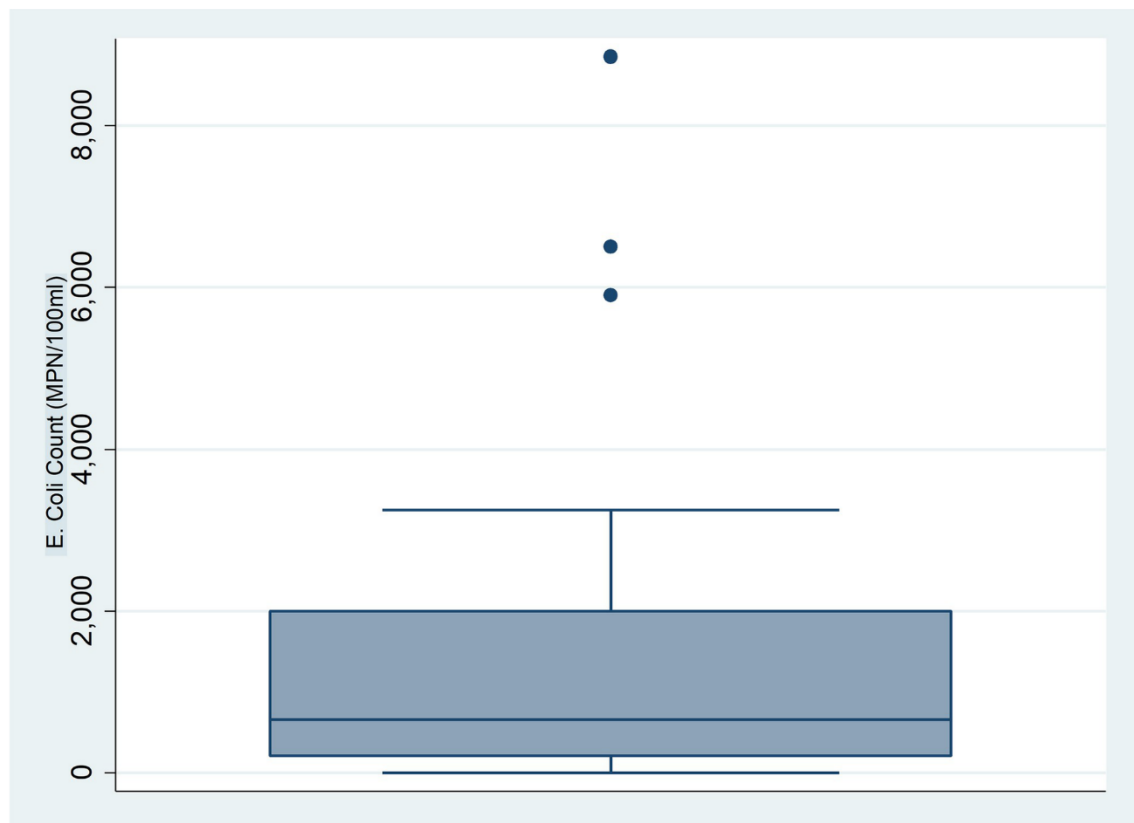


Fig 1. Box plot showing *E. coli* count in groundwater sources.

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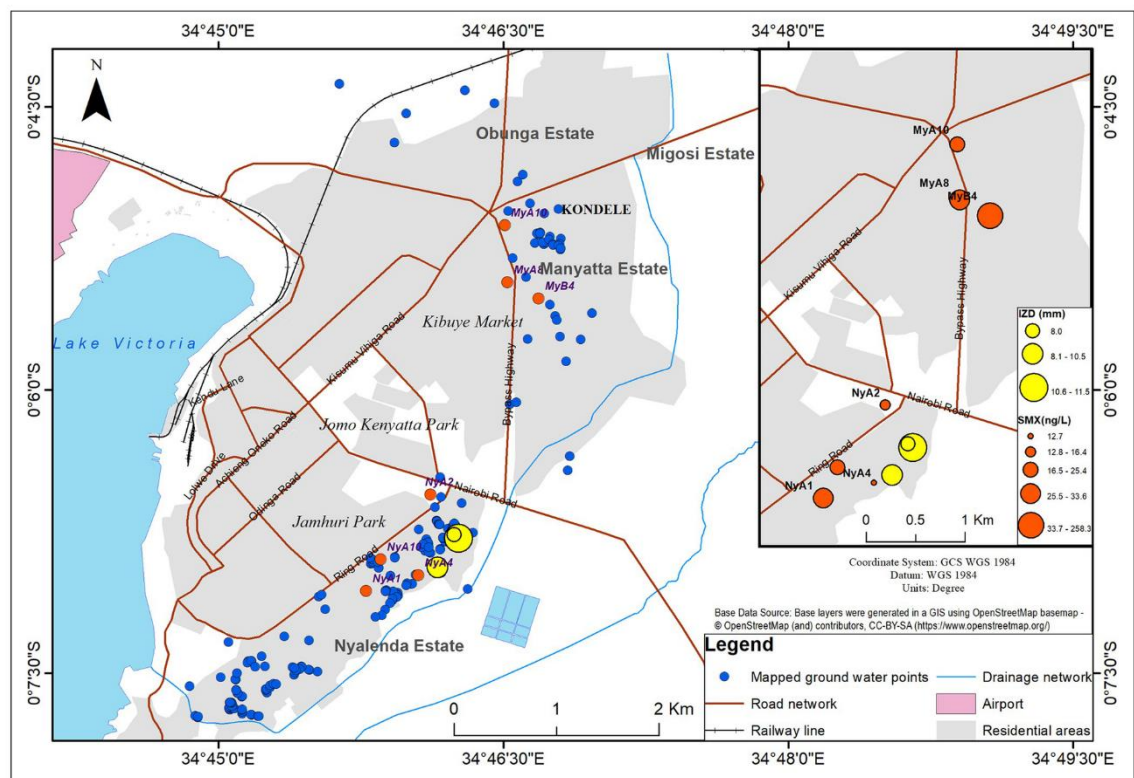


Fig 2. Map of study area showing the distribution of mapped groundwater points (blue), groundwater points where sulfamethoxazole was detected (orange) and groundwater sources where antibiotic resistant *E. coli* was detected (yellow).

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During data collection, water quality was assessed by measuring the pH of the water samples with a HydroLab QUANTA multi parameter water quality meter, Model No. 16245QD04652.

**2.1.2 Chemicals and standards.** Water samples were kept at  $-4^{\circ}\text{C}$  in sterile water bottles. The water samples were filtered with Agilent Technologies glass fiber filters (1 m) and PTFE/nylon filters (0.2 m) before analysis. All antibiotic standards bought from Sigma Aldrich and were of high purity ( $>90\%$ ). For each of the two analytes, a 1000 mg/l mixed standard stock solution was produced in methanol and water (50:50, v/v). Following preparation, the stock solution was kept at  $-80^{\circ}\text{C}$ . (KEBS Protocol- August 16th 2021). The spiked samples for recovery yield analysis were generated by adding 10 L, 125 L, and 250 L of the 1 ppm stock solution into 250 ml of water, yielding final concentrations of 10  $\mu\text{g/kg}$  (ppb), 50  $\mu\text{g/kg}$ , and 100  $\mu\text{g/kg}$ , respectively. S1 Table shows the instrument conditions of the Solid Phase Extraction and Liquid Chromatography (SPE-LC-MS/MS) used in the analysis of groundwater samples.

Agilent Technologies' Agilent Bond elut Plexa cartridges (60 mg, 3 ml; Part number: 12109603) were used for solid phase extraction. Liquid chromatography-mass spectrometry (LC-MS) grade methanol, acetonitrile, water, and formic acid 98% were purchased from Merck (Darmstadt, Germany). EDTA and Hydrochloric acid 37% were purchased from Sigma-Aldrich, and nitrogen for drying was generated in the laboratory.

**2.1.3 Sample processing and analysis.** Water sample clean-up and pre-concentration was done following the protocol used by Rodriguez-Mozaz et al. [32] and modified to use an automated Solid Phase Extraction system (SPE).

Water was filtered using 1 µm fibre filters, followed by 0.45 µm PTFE/nylon filters. To the filtered water, a 0.1M solution of sodium ethylenediaminetetraacetic acid (EDTA) was added to achieve a final concentration of 1g/L (g solute/g solution) in addition to 0.1M hydrochloric acid which was added to correct the pH to 2.5. The samples were then filtered via a Whatman filter paper into a 250 ml conical flask.

Solid phase extraction (SPE) was performed according to Rodriguez-Mozaz et al., (2020). Briefly, Agilent Bondelut Plexa cartridges (60 mg, 3 ml; Part number: 12109603) were first conditioned with 6 ml methanol followed by 6 ml deionized water (UPLC grade, and pH adjusted to 2.5 with HCl 0.1 M). A portion of 100 ml of the sample was passed through the SPE cartridge sample at approximately 1 mL/min. Each cartridge was cleaned with 6 ml HPLC water (under gravity conditions) and dried under vacuum conditions for 10–15 min. For analyses, the samples were eluted from the cartridge with 6 ml of ultra-pure methanol, evaporated under N<sub>2</sub> stream to near dryness, and reconstituted with 1 mL of mixture of methanol and water (50:50, v/v). Recovery values of the extraction method were calculated in each occasion (each sampling campaign) and used to correct the quantification values obtained using these calibration curves. To determine the recoveries, samples were spiked in triplicate with a standard mixture containing all antibiotics at a final concentration of 10 ng/L. Recoveries were determined by comparing the initial concentrations after spiking with the concentrations obtained after the whole SPE procedure. Method detection limits (MDL) and method quantification limits (MQL) were also determined in each sampling campaign and for each antibiotic as the minimum detectable amount of compound with a signal-to-noise ratio of 3 and 10, respectively.

The Agilent 6460 Triple Quadrupole MS (Model K6460) is a liquid chromatograph that uses three sets of parallel rods (quadrupole, hexapole and quadrupole) to perform tandem mass spectrometry (MS-MS). The first quadrupole separates ions into precursor ions which are then fragmented in the hexapole into product ions, which are separated by the second quadrupole.

## 2.2 Isolation of *Escherichia coli* and determination of antibiotic resistance

An enzyme substrate was used to identify *E. coli*. Colilert Test, which detects coliforms and *Escherichia coli* concurrently using proprietary Defined Substrate Technology (DST). The test consisted of two nutritional indicators that were the principal sources of carbon colilert and were digested by the *Escherichia coli* form enzymes -galactosidase and -glucuronidase, respectively. In a sterile glass bottle, 100ml of water sample was mixed with test reagent and allowed to stand for 10 minutes (until the reagent dissolved).

After that, the mixture was placed into a tray and the contents were sealed with a Quanti tray sealer. This was kept in an incubator for 24 hours at a temperature of 35°C ±0.5°. The wells were counted and positive samples were detected by their fluorescent color. To estimate the *Escherichia coli* count, the Most Probable Number table was used.

The confirmed isolates were then cultured further in nutrient agar plates to generate well isolated colonies. These isolated colonies were diluted further in normal saline. The inoculated organisms' sizes were standardized using McFarland's 0.5 solution. Colonies were grown and picked from the samples before being transferred to 3ml of sterile distilled water to prepare a bacterial suspension. Portions of 100µL of the suspension were distributed on Muller Hinton agar and incubated at 37° C for 24 hours Using the Kirby-Bauber disk diffusion method, a

Table 1. The multiple reaction monitoring parameters.

| Target Compound | RT <sup>a</sup> | Precursor Ion [M + H] <sup>+</sup><br>(m/z) (CV) <sup>b</sup> | Quantifier Ion<br>(m/z) (CE) <sup>c</sup> | Qualifier Ion (CE) |
|-----------------|-----------------|---------------------------------------------------------------|-------------------------------------------|--------------------|
| SMX             | 4.74            | 254 (110)                                                     | 156 (12)                                  | 108 (24)           |
| TMP             | 4.24            | 291.1 (60)                                                    | 123.1 (30)                                | 80.9 (25)          |

<sup>a</sup>RT—retention time.<sup>b</sup>CV collision voltage.<sup>c</sup>CE collision energy.

SMX = Sulfamethoxazole, TMP = Trimethoprim.

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pure culture of *Escherichia coli* bacterium was tested for antibiotic susceptibility to Sulfamethoxazole and Trimethoprim [33, 34]. To define antibiotic resistance, Antibiotic Inhibition Zone Diameters (IZD) were measured and recorded. Bacterial susceptibility was measured by their capacity to grow around a thin material containing antibiotics. The area where bacteria did not grow was apparent and referred to as a zone of inhibition; the larger the zone, the more susceptible the bacteria, and the smaller the zone, the less susceptible the bacteria, signifying resistance.

### 3. Results

The reaction monitoring parameters for the three analytes were as shown in Table 1.

Method quantification results were as shown in Table 2.

#### 3.1 Occurrence of antibiotics in groundwater

Detection frequency for sulfamethoxazole in groundwater sources in the informal settlements of Kisumu was 14.3% (n = 7). This was out of 49 groundwater samples, with concentrations ranging from no detection (nd) to 258.21 ng/L (Table 3). All the water samples were from shallow wells. Trimethoprim was however below the detection limit in groundwater.

The average concentrations of sulfamethoxazole in groundwater are summarized in Table 4. Sulfamethoxazole was detected in 7 out of 49 groundwater points with the levels ranging from a minimum concentration of 12.7 ± 1.1 ng/L and a maximum concentration of 258.2 ± 27.4 ng/L (SD = 27.440).

The mapped groundwater points studied in the informal settlements were

Table 2. LC-MS/MS-ESI method quantification results.

| API              |               | r <sup>2</sup> | % recovery | SD    | RSD   | Repeatability | LOQ (ng/L) |
|------------------|---------------|----------------|------------|-------|-------|---------------|------------|
| Trimethoprim     |               | 0.983          |            |       |       |               | 55.89      |
|                  | Spike 10 ppb  |                | 112.7      | 1.69  | 1.5   | 5.19          |            |
|                  | Spike 50 ppb  |                | 99.13      | 12.95 | 13.07 | 39.92         |            |
|                  | Spike 100 ppb |                | 100.23     | 4.93  | 4.92  | 15.18         |            |
| Sulfamethoxazole |               | 0.9897         |            |       |       |               | 108.25     |
|                  | Spike 10 ppb  |                | 106.83     | 4.62  | 4.32  | 14.24         |            |
|                  | Spike 50 ppb  |                | 111.8      | 2.15  | 1.92  | 6.63          |            |
|                  | Spike 100 ppb |                | 111.79     | 9.25  | 8.28  | 28.51         |            |

SD: standard deviation, RSD: Relative standard deviation.

API = Active pharmaceutical ingredient, ILIS = Isotopically labelled internal standard, LOQ = limit of quantification, SMX = sulfamethoxazole, TMP = trimethoprim.

<https://doi.org/10.1371/journal.pwat.0000076.t002>

Table 3. Detection frequency of antibiotics in groundwater.

| Antibiotic       | Detection frequency (%) | Range (ng/L) |
|------------------|-------------------------|--------------|
|                  | (n = 49)                |              |
| Sulfamethoxazole | 14.3                    | nd- 258.21   |
| Trimethoprim     | nd                      |              |

nd: not detected.

<https://doi.org/10.1371/journal.pwat.0000076.t003>

### 3.2 *Escherichia coli* count

The *E. coli* count was determined and the microbe was present in 98% (n = 48) groundwater samples. The count ranged from 16 to 8,850 MPN/100ml. The mean was 1245.77 MPN/100ml and a standard deviation of 1726.01. The majority of the observations were in the third quartile as shown in Fig 1 the box plot of the *E. coli* count (MPN/100ml).

Antibiotic sensitivity tests revealed antibiotic resistance in three (3) groundwater points, representing about 6% of all the groundwater sources sampled; with Inhibition Zone Diameters (IZD) of 0.8mm (resistant), 10.5mm (resistant), and 11.5mm (intermediate), but none of the three samples were among samples where SMX was detected. Water sources where antibiotic sulfamethoxazole was detected and the water points where resistant bacteria were detected were placed on the map of the study area as shown in Fig 2.

## 4. Discussion

Sulfamethoxazole and trimethoprim were analyzed in groundwater. These antibiotics usually used in a fixed-dose combination and are recommended for the prevention and treatment of opportunistic infections such as urinary tract infections caused by *Escherichia coli* and Pneumocystis pneumonia caused by *Pneumocystis jirovecii* in HIV/AIDS patients as well as in the general population [35] (WHO, 2016). The antibiotic is widely accessible in resource constrained settings live informal settlements, is well tolerated and an inexpensive intervention in the reduction of morbidity and mortality among HIV/AIDS patients [35] (WHO, 2016). Cotrimoxazole is also recommended by Kenya's Ministry of health, for management of opportunistic infections that include malaria in people living with HIV/AIDS [36]. The fact that this study site is in a region that reports a prevalence of HIV; between 16.3% and 17.5% which is higher than the national prevalence, it is an indication that use of the antibiotic is likely to be high and thus likely to pollute the environment, including groundwater. A study by [37] reported that about 29% of decedents at the high volume mortuaries were reported to have died of HIV/AIDS, an indication of the burden of disease and related mortality. Cotrimoxazole is also

Table 4. Concentration of sulfamethoxazole in groundwater samples.

| Code  | Sample | SMX (ng/L) | SD   |
|-------|--------|------------|------|
| NyA4  | 1      | 12.7       | 1.1  |
| MyA10 | 6      | 25.4       | 10.6 |
| MyA8  | 9      | 33.6       | 9.5  |
| MyB4  | 10     | 258.2      | 27.4 |
| NyA10 | 14     | 24.6       | 1.1  |
| NyA2  | 18     | 16.4       | 0    |
| NyA1  | 20     | 29.9       | 8.4  |

SMX: sulfamethoxazole SD: standard deviation.

<https://doi.org/10.1371/journal.pwat.0000076.t004>

indicated for malaria prevention [36], and the study area also reports a high malaria prevalence, thus contamination levels are likely to be greater. Contamination of groundwater with these antibiotics is unacceptable as it jeopardizes treatment effectiveness. This is possible since groundwater consumption has increased over time to complement the unstable source of water from the local county authority [31], where they also rely on chlorination and boiling to purify water [38].

Because of the increased population density in informal settlements, antibiotic use is bound to be high since in informal settlements, the prevalence of infectious diseases is known to be high. This is known accelerate contamination of groundwater and in effect spread of antibiotic resistance due to the rise in the number of on-site sanitation facilities like pit latrines [31]. There is likely interaction of the aquifer and sanitation facilities due to the limited construction depth of both pit latrines and shallow wells in the study site [29, 39]. The Kisumu drainage network and the wastewater treatment site are close to the sites where antibiotic resistant *E. coli* were isolated, indicating a possible contamination in the sub-surface. Resident of the informal settlements do not rear animals on large scale that could contribute to contamination of the environment with antibiotics for veterinary use, but the source should not be underestimated. Furthermore, there is rising concern about the increasing impact of climate change on groundwater quality in shallow aquifers [40]. Studies on pharmaceutical contamination of groundwater are on the rise, which warrants attention because the effect of pollution has not been thoroughly studied.

Even in small doses, antibiotics can affect the human body, animals, and the environment. According to a review by [41], antibiotics are prevalent in the environment, presenting a risk of accumulation in soil and infiltration into drinking water. The value ranged from 0.1 to 1,110 ng/L in natural water samples analyzed by reviewed research, which by far exceeded (220x) the safe limit for researched organisms. Because continual antibiotic release endangers the ecology and aquatic organisms, it imperative to discourage continuous release into the environment.

The detection frequency of sulfamethoxazole in groundwater sources in Kisumu's informal settlements was 14.3%, with values ranging from no detection (nd) to 258.21 ng/L. This detection frequency of SMX can be related to its high mobility in soil due to qualities such as its relatively small molecular size (mass 253.3 Da), weak sorption, and inability to rapidly biodegrade due to stability in water as well as soil and physicochemical properties [42, 43]. Trimethoprim, on the other hand was found to be below the detection limit in groundwater, but then a study in two shallow wells in the Kisumu's densely populated informal settlements by K'Oreje et al. 2015 in September 2021 found SMX and TMP in concentrations ranging from 5 to 50 ng/L [44], but acknowledges that the compounds are detected sporadically in groundwater. This differs from our investigation, in which TMP was absent but SMX was present in higher proportions, and could be attributable to probable hydraulic events associated with groundwater and surface water fluctuations. According to a study conducted in Lusaka, Zambia, sulfamethoxazole has a greater detection frequency in water matrices than other pharmaceutical chemicals under examination [9] which was attributed to its low solubility in water [45].

A recent investigation on environmental contamination in a high-income country (France) found SMX to be the most frequently identified antibiotic, with detection rates as high as 27% and concentrations as low as 5 ng/L in groundwater sources [46]. An environmental monitoring of antibiotics in surface-groundwater on the other hand did not detect SMX in groundwater but TMP was detected in 2 out of 7 (29%) groundwater sources [47]. This Portuguese case study that tracked pharmaceutical compounds discovered a considerably higher concentration of antibiotics downstream of effluent discharge compared to upstream, implying low treatment plant performance and hence a possible source of surface-groundwater pollutants [47].

A study on sulfamethoxazole pollution of a deep phreatic aquifer in Tel Aviv discovered SMX in all investigated wells (highest concentration was 37 ng/L), with a concentration of up to 20ng/L in the water table region [48]. An evaluation of chemical tracers in suburban groundwater as indications of nitrate-nitrogen sources detected sulfamethoxazole at a low concentration of 0.025ng/L in shallow wells, indicating contamination with septic tank effluent [49]. The Baix Fluvia alluvial aquifer in Catalonia, Spain, had a greater detection frequency for SMX in ground water (>80%), with values that did not surpass 10ng/L, but TMP was not found in any of the collected samples [50]. Nguyen et al., 2015 found none of the antibiotics under inquiry in groundwater in Vietnam's Mekong Delta, however they were detected in surface water used for drinking at median quantities of 21ng/L sulfamethoxazole (SMX) and 17ng/L trimethoprim (TMP) [51]. This was therefore, considered to have limited risk to aquatic ecosystems since the concentrations were lower than the predicted no effect concentrations (PNECs) and minimum inhibitory concentrations (MICs) [51]. Antibiotic transformation metabolites, as well as other environmental factors such as aquifer vulnerability and groundwater pH, may all have a role in transfer of contaminants and antibiotic resistance [24, 50, 52].

Trimethoprim, on the other hand, was not discovered in any of the samples, possibly due to its high distribution co-efficient ( $pK_a, 7.12$ ) [52]. The pH of the water which was between 6.4 and 8.3 had no effect on TMP unavailability. However, a research in Lusaka, Zambia, found that lowered soil pH from between 4.02 to 5.56 was within the TMP optimal sorption range, rendering TMP immobile and lowering detection frequency (34.6%) and concentrations with the maximum being 140ng/L [9]. TMP is generally inaccessible in groundwater due to its greater molecular weight of 290.32 Da and its weak mobility and solubility in water [47], although it was identified in Portugal in 2 out of 7 (29%) groundwater sources. Furthermore, SMX and TMP are used in combination, with each pill containing 80mg Trimethoprim BP and 400 mg Sulfamethoxazole BP in a 5:1 ratio [35]. TMP sorption qualities could also be explained by the hydrogeology of the research region, where the soil is clayey with high porosity but low permeability [4]. However, the underlying layer is composed of less weathered, partially altered rocks with better permeability [4].

The study area is known to post high HIV prevalence that is higher than the national figure, raising an alarm on the consequences of exposure and threat of resistance in this population. In addition to exacerbating antibiotic resistance, increased antibiotic use has been found to increase cost of health care and increased risk of adverse reactions to drugs [53]. Treatment failure in patient who already have a compromised immune system is a real burden to the patients, in addition to the fact that they already suffer inequities in their daily lives.

There is substantial data on antibiotic contamination of surface water, but data on groundwater contamination is scarce, particularly in developing nations and informal settlements. The levels of sulfamethoxazole, a component of cotrimoxazole, reported in this study are of environmental concern. The disparity in detection could be attributed to the fact that the SMX ratio in the combination is higher (4:1) than that of trimethoprim [9]; however, the fate of TMP in groundwater should be carefully investigated to determine the fate in the environment because the development of resistant pathogens that can be transferred from environmental pathogens to human pathogens can be a one-time event [54]. It is vital to have a solid understanding of the fate of pharmaceutical pollutants, particularly antibiotics from sanitation facilities such as pit latrines and septic tanks that share the subsurface with shallow aquifers. This is due to the reliance on groundwater for domestic use and the related risk of antibiotic resistance development. As a result, it is critical to begin conversations about antibiotic use and disposal.

*Escherichia coli* has been found in a variety of water sources, including those used for drinking [55], and linked to informal settlements and environmental deterioration [18]. This is a

challenge since environmental degradation is linked to exposure to calamities like flooding. A study in Bangladesh found *Escherichia coli* and fecal coliforms in drinking water sources and household point of use, which was attributed primarily to inadequate hygiene [56]. This study's findings are also consistent with those of a 2012 study conducted in the same Kisumu informal settlements, which found that up to 95% (n = 76) of the sampled water points were highly contaminated with *E. coli*; all water samples from unprotected wells and 92.6% (n = 25) of the water from protected wells were positive for *E. coli*, with low densities observed from dams and boreholes [19]. A research conducted in Kinsasha's sub-rural areas discovered high levels of *E. coli* contamination in groundwater and recreational water, which was connected to inadequate sanitation [57].

Antibiotic resistance is a significant worldwide health problem, with rates increasing both in the environment and in clinical settings. A review of groundwater resources as a global reservoir for antimicrobial resistant bacteria discovered up to 80% of groundwater isolates resistant to more than one antimicrobial, as well as antibiotic resistant genes identified in wells and springs, but recommended additional research to understand the mechanisms that trigger resistance in the environment [58]. *Escherichia coli* isolated from water samples from drinking water sources in northern Tanzania was resistant to one or more antibiotics, with resistance to ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim being greater than resistance to other antibiotics [59]. These are the antibiotics that are used as the first line of treatment for any bacterial illness. A study on antibiotic-resistant *Escherichia coli* in drinking water among households in Cajamarca, Peru found the highest resistance to antibiotics, among them sulfamethoxazole-trimethoprim (21.4%), implying that potential environmental contaminants should be studied to identify potential sources of contamination, misuse, or inadequate antibiotic disposal [60].

The presence of antibiotics in groundwater indicates that the shallow aquifer in the research area is at risk of contamination with other contaminants that are hazardous to human health. This is threat to the people living in informal settlements, considering they already have challenges of coping with limited resources available for use in the crowded space.

## 5. Conclusions and recommendations

The presence of antibiotics in groundwater indicates that the shallow aquifer in the research area is at risk of contamination with other contaminants that are hazardous to human health. The 2019 Kenya census report shows an increase in population, as seen by the shift in the number of households in informal settlements (KNBS, 2010; KNBS, 2019). This is an indication of rising population density, which leads to increased demand for services such as sanitary facilities, which are expected to contribute to groundwater contamination.

Informal settlements are known to have elevated prevalence of infectious diseases and therefore antibiotic use is bound to be higher. Contamination of groundwater and antibiotic resistance poses a challenge of provision of improved quality of life among people living in informal settlements. Given the increased population growth and the projected AMR crisis, sustainable solutions need to be found to reduce the contamination of the water sources and spread of resistance in the environment. The problem should be addressed at the point of antibiotic use, where both prescribers and users should be sensitized on safe antibiotic prescription. This, together with further collaborative research involving all players concerned with water provision to establish presence of APIs and interactions will be critical in shielding the ecosystem from the impacts of sub-optimal dosing of a medicinal substance or its metabolites.

With prior knowledge of the HIV disease factor in the research site, additional research to determine the presence of cotrimoxazole in all groundwater sources throughout the study area

is required to better understand the spread of antibiotic resistant *Escherichia coli*. The soil conditions that cause trimethoprim non-detection in groundwater should also be explored.

### 5.1 Study limitation

Because this was an academic study, budgetary constraints prevented the collecting of multiple samples.

### Supporting information

**S1 Table. Instrument conditions of the solid-phase extraction and liquid chromatography (SPE-LC-MS/MS) use in the analysis of the groundwater samples.**  
(DOCX)

**S1 Data.**  
(PDF)

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## RESEARCH PRESENTATIONS

### Research dissemination

# Investigation of contamination of groundwater sources with antibiotics in informal settlements of Kenya

Kellen Karimi

University of Nairobi

Department of Public and Global Health

29.03.2022



### Conference presentation



*Emerging Pollutants: Protecting Water Quality for the Health of People and the Environment*

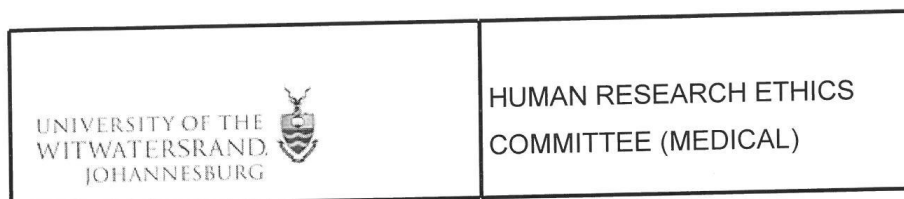
**Presentation Title: Groundwater contamination with Sulfamethoxazole and trimethoprim and spread drug resistant of E.coli in informal settlements; Kenya**

**Ms. Kellen Joyce Karimi**

*18<sup>th</sup> January 2023*



## ETHICAL APPROVAL



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**CC:** Supervisor: Dr A Ahmad <[Aijaz.Ahmad@wits.ac.za](mailto:Aijaz.Ahmad@wits.ac.za)>  
and <[HREC-Medical.ResearchOffice@wits.ac.za](mailto:HREC-Medical.ResearchOffice@wits.ac.za)>

**FROM:** Iain Burns  
Human Research Ethics Committee (Medical)  
Tel: 011 717 1252

E-mail: [Iain.Burns@wits.ac.za](mailto:Iain.Burns@wits.ac.za)

**DATE:** 2019/07/16

**REF:** R14/49

**PROTOCOL NO:** M190412 *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

**PROJECT TITLE:** *Investigation of contamination of community groundwater sources with antibiotics in informal settlements in Kisumu, Kenya*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps



R14/49 Ms KJ Karimi

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) CLEARANCE CERTIFICATE NO. M190412

**NAME:** Ms KJ Karimi  
**(Principal Investigator)**  
**DEPARTMENT:** School of Pathology  
 Dept of Clinical Microbiology & Infectious Diseases  
 Medical School  
 University

**PROJECT TITLE:** Investigation of contamination of community groundwater  
 sources with antibiotics in informal settlements in  
 Kisumu, Kenya

**DATE CONSIDERED:** 2019/04/26

**DECISION:** Approved unconditionally

**CONDITIONS:**

**SUPERVISOR:** Dr A Ahmad

**APPROVED BY:**

  
 Dr CB Penny, Chairperson, HREC (Medical)

**DATE OF APPROVAL:** 2019/07/16

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

### **DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.  
 I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **April** and will therefore reports and re-certification will be due early in the month of **April** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

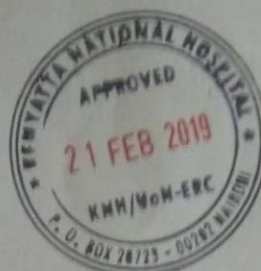
Principal Investigator Signature

Date

**PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES**



UNIVERSITY OF NAIROBI  
COLLEGE OF HEALTH SCIENCES  
P O BOX 19676 Code 00202  
Telegrams: varsity  
Tel: (254-020) 2726300 Ext 44355



KNH-UoN ERC  
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Website: <http://www.erc.uonbi.ac.ke>  
Facebook: <https://www.facebook.com/uonknh.erc>  
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KENYATTA NATIONAL HOSPITAL  
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Tel: 726390-9  
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Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/59

21 February, 2019

Kellen J. Karimi  
PhD Candidate  
Dept. of Clinical Microbiology and Infectious Diseases  
Faculty of Health Sciences  
University of the Witwatersrand, South Africa  
Email: [karimikellen@gmail.com](mailto:karimikellen@gmail.com), [kkarimi@cartafrica.org](mailto:kkarimi@cartafrica.org)

Dear Kellen

**RESEARCH PROPOSAL – INVESTIGATION OF CONTAMINATION OF COMMUNITY GROUNDWATER SOURCES WITH ANTIBIOTICS IN INFORMAL SETTLEMENTS IN KISUMU, KENYA (P719/10/2018)**

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 21<sup>st</sup> February 2019 – 20<sup>th</sup> February 2020.

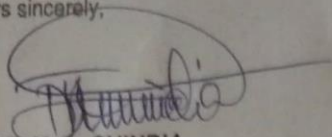
This approval is subject to compliance with the following requirements:

- Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
- Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
- Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
- Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
- Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
- Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website <http://www.erc.uonbi.ac.ke>

Protect to discover

Yours sincerely,



**PROF. M. L. CHINDIA**  
**SECRETARY, KNH-UoN ERC**

- c.c. The Principal, College of Health Sciences, UoN  
The Director, CS, KNH  
The Chairperson, KNH- UoN ERC  
The Assistant Director, Health Information, KNH  
Supervisors: Dr. Aijaz Ahmad, Dept. of Clinical Microbiology & Infectious Diseases, University of Witwatersrand  
Prof. Adriano G. Duse, Dept. of Clinical Microbiology & Infectious Diseases, University of Witwatersrand  
Prof. Mutuku Mwanthi, School of Public Health, UoN

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TECHNOLOGY AND INNOVATION**

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NACOSTI, Upper Kabete  
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P.O. Box 30623-00100  
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/3232/28732**

Date: **14<sup>th</sup> March, 2019**

Prof. Daniel Ochieng' Olago  
University of Nairobi  
P.O. Box 30197-00100  
**NAIROBI**

**RE: RESEARCH AUTHORIZATION**

Following your application for authority to carry out research on "*AFRIWATSAN - Sustaining Low Cost Urban Water Supplies and Sanitation Systems in Africa*" I am pleased to inform you that you have been authorized to undertake research in **Kisumu County** for the period ending **12<sup>th</sup> March, 2020**.

You are advised to report to **the County Commissioner and the County Director of Education, Kisumu County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

**GODFREY P. KALERWA MSc., MBA, MKIM  
FOR: DIRECTOR-GENERAL/CEO**

Copy to:

The County Commissioner  
Kisumu County.

The County Director of Education  
Kisumu County.

## ORIGINALITY REPORT

# INVESTIGATION OF CONTAMINATION OF COMMUNITY GROUNDWATER SOURCES WITH ANTIBIOTICS IN INFORMAL SETTLEMENTS OF KISUMU, KENYA

## ORIGINALITY REPORT

|                  |                  |              |                |
|------------------|------------------|--------------|----------------|
| <b>19%</b>       | <b>6%</b>        | <b>17%</b>   | <b>2%</b>      |
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## PRIMARY SOURCES

|          |                                                                                                                                                                                                                                                                            |           |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>1</b> | Kellen J. Karimi, Aijaz Ahmad, Adriano Duse, Mutuku Mwanthi, Richard Ayah. "Prevalence of Antibiotic Use and Disposal at Household Level in Informal Settlements of Kisumu, Kenya", International Journal of Environmental Research and Public Health, 2022<br>Publication | <b>3%</b> |
| <b>2</b> | Sara Rodriguez-Mozaz, Ivone Vaz-Moreira, Saulo Varela Della Giustina, Marta Llorca et al. "Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment", Environment International, 2020<br>Publication     | <b>1%</b> |
| <b>3</b> | <a href="http://www.agilent.com">www.agilent.com</a><br>Internet Source                                                                                                                                                                                                    | <b>1%</b> |
| <b>4</b> | <a href="http://www.ncbi.nlm.nih.gov">www.ncbi.nlm.nih.gov</a><br>Internet Source                                                                                                                                                                                          | <b>1%</b> |

**SIMILARITY INDEX MOTIVATION****To: Postgraduate Office****Ref:** 2012904, Kellen J Karimi PhD Thesis**Re.: Motivation for high Similarity Index report**

I am writing this letter to bring to your attention that the similarity index of my Phd Thesis is 19%. As per the Turnitin report, out of 19% similarity index, 3% is from our previously published article from the thesis. It is worth noting that this is expected as the Turnitin records it as a plagiarism as the paper is published.

The other reason of high similarity index is attributed to commonly used phrases in the microbiological community and the names of microorganisms. It is essential to acknowledge that these similarities are unavoidable in scientific writing and should not be considered as plagiarism.

Please note that this work meets all the necessary academic standards, and the thesis should be evaluated based on its scientific merit and contribution to the field of microbiology.

Should you require any further information or clarification regarding this matter, please do not hesitate to contact me.

Sincerely,

Kellen J Karimi

Student Number: 2012904