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
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 km² and administers 567 km² 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.
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°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°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 μg/kg (ppb), 50 μg/kg, and 100 μg/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 1μm 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 N₂ 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* 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°C. 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
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...
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.

### 3.2 *Escherichia coli* count

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### 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. Cotrimxazole is also

### Table 3. Detection frequency of antibiotics in groundwater.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Detection frequency (%)</th>
<th>Range (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td>14.3</td>
<td>nd- 258.21</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

nd: not detected.

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### Table 4. Concentration of sulfamethoxazole in groundwater samples.

<table>
<thead>
<tr>
<th>Code</th>
<th>Sample</th>
<th>SMX (ng/L)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NyA4</td>
<td>1</td>
<td>12.7</td>
<td>1.1</td>
</tr>
<tr>
<td>MyA10</td>
<td>6</td>
<td>25.4</td>
<td>10.6</td>
</tr>
<tr>
<td>MyA8</td>
<td>9</td>
<td>33.6</td>
<td>9.5</td>
</tr>
<tr>
<td>MyB4</td>
<td>10</td>
<td>258.2</td>
<td>27.4</td>
</tr>
<tr>
<td>NyA10</td>
<td>14</td>
<td>24.6</td>
<td>1.1</td>
</tr>
<tr>
<td>NyA2</td>
<td>18</td>
<td>16.4</td>
<td>0</td>
</tr>
<tr>
<td>NyA1</td>
<td>20</td>
<td>29.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>

SMX: sulfamethoxazole SD: standard deviation.

[https://doi.org/10.1371/journal.pwat.0000076.t004](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 258.21ng/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 5ng/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 groundwater (>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 (pKa,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 Kinshasa’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 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.

S1 Data.

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References


