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Assessment of resistant *Escherichia coli* in groundwater sources and sanitary inspection for contamination risk in Bagamoyo, Tanzania

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Abstract

Two billion people globally are using faecal-contaminated water. Consumption of *Escherichia coli* in fecal-contaminated waters poses a health risk due to its potential to cause diseases which highlights the need for understanding its factors for contamination and its antimicrobial resistance profile. Membrane filtration method was used to culture and isolate *E. coli*. The disk diffusion method was used to identify resistance of *E. coli* isolated and sanitary inspection was used to identify the contamination risk. The study found 44.8% of groundwater samples (n=73) were contaminated with *E. coli*, with 83.6% (n=61) of isolates showing antibiotic resistance. A Chi-square test revealed a statistically significant difference in resistance proportions across tested antibiotics ($p < 0.001$). High resistance rates were noted for cefazolin (56.16%, n=41), nitrofurantoin (54.79%, n=40), amoxicillin-clavulanic acid (45.21%, n=33), and ceftriaxone (42.47%, n=31). Additionally, 70% of *E. coli* isolates (n=51) were multidrug-resistant. Sanitary inspections showed 46.2% (n=18) of dug wells had a high sanitary risk, while 73.3% (n=44) of boreholes had a low contamination-risk. However, even boreholes with a low sanitary risk showed a high rate of *E. coli* contamination. Tube wells with hand pumps and dug wells at high sanitary risk also had elevated rates of *E. coli* contamination. Findings showed high prevalence of antibiotic-resistant *E. coli* in groundwater in Bagamoyo, and highlight need for advocacy of proper use of antibiotics and proper hygiene to prevent the spread of resistant *E. coli* in groundwater.

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Introduction

Globally, it is estimated that two billion people are using faecal-contaminated water, most of which are from developing countries according to WHO and UNICEF joint monitoring program for water supply of 2020. The most frightening issue is that people drink this contaminated water, which contains *Escherichia coli* that threaten their health (*E. coli*). *Escherichia coli* was reported as one of the bacteria which causes diarrheal disease especially in areas with poor sanitation especially in developing countries (WHO, 2019). It has been reported that diarrheal disease is responsible for 370,000 children's annual mortality worldwide (Pokharel *et al.*, 2023). In addition, *E. coli* causes peritonitis, pneumonia, urinary tract infections (UTIs), and urinary bacteremia (Fan *et al.*, 2023; Sinnott-Stutzman and Sykes, 2021). Despite causing such diseases, recent emerging concern globally is increasing antimicrobial resistance, a top ten public health threat resulting from antibiotic overuse for human treatments, in animals, food production, and discharge to the environment (Kusi *et al.*, 2022; Salam *et al.*, 2023).

Antimicrobial resistance develops when bacteria, viruses, fungi, and parasites evolve and become resistance to antimicrobial agent which making infections more difficult to treat and raising the risk of disease spread, severe illness, and death (Tang *et al.*, 2023). Notably, antibiotic resistance occurs through three primary mechanisms: antibiotic drug degradation by enzymes, change of bacterial proteins targeted by antibiotics, and alteration of the membrane permeability to antibiotics (Darby *et al.*, 2023). In addition, extended-spectrum beta-lactamase (ESBL), a plasmid-mediated lactamase capable of hydrolyzing and deactivating beta-lactams like cephalosporin and monobactams is a method by which *E. coli* gain resistance to beta-lactam antibiotics (Sawa *et al.*, 2020). Although all enzymatic effects to maximize the ease of binding to their target sites can inactivate antibiotics, *E. coli* uses outlet pumps or alters membrane permeability to bring the most valuable amount of drug closer to the cell (Arbab *et al.*, 2022).

It is estimated that by the year 2050, antimicrobial-resistant pathogens are predicted to kill 4.2 million people annually in Africa, with most of those deaths expected to occur in Sub-Saharan Africa (SSA) if the current trend holds (Gwenzi *et al.*, 2022; Sulis *et al.*, 2022). A global scoping review (Chique *et al.*, 2021) and other studies undertaken have related the risks of antimicrobial resistance with water sources and reported an increase in *E. coli* resistance to antibiotics in water sources from different countries in Africa (Babatunde *et al.*, 2022; Ramatla *et al.*, 2023). *Escherichia coli* has been reported as the leading cause of death due to antimicrobial resistance followed by *Staphylococcus aure*us, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginos*a (Murray *et al.*, 2022). This highlights the need to investigate antibiotic resistance in Tanzania to understand the extent of possible contaminated water sources and identify human infection hotspots. This will assist in planning control mechanisms and help prevent the spread of resistant bacterial infections in regions depending on groundwater as alternative source non-availability of pipe water supply (Ligate *et al.*, 2021).

Bagamoyo district relies on groundwater sources, including dug wells, tube wells with hand pumps or boreholes as an alternate supply and various studies reveal that these water sources are affected by physiological features, hydrology, and soil texture (Zhang *et al.*, 2022). In a simulation study examining the use of alternative unimproved water sources, they discovered a 95% increase in annual exposure to *E. coli*, which WHO has identified as a highly significant and representative indicator of its greatness, leading to the worldwide antimicrobial resistance problem (Daly and Harris, 2022; Loayza *et al.*, 2020). Due to rising concern about the prevalence of antibiotic resistant *E. coli* in drinking water sources in low-income countries (Ahmed *et al.*, 2022), it is critical to investigate the exposure of groundwater sources to antibiotic-resistant *E. coli*, because antibiotic-resistant genes can be vertically transported and horizontally propagated among bacteria through mobile genomic elements (Von Wintersdorff *et al*., 2016). This highlights the importance of monitoring and understanding the spread of antibiotic resistance to effectively combat its potential impact on public health. Therefore, this study aims to determine the prevalence of resistant *E. coli* and the sanitary contamination risk level of groundwater sources from sanitary facilities in Bagamoyo District, Tanzania.

Materials and methods

Study area

This study was conducted in Bagamoyo District Council, located at 6.4428° S and 38.9085° E in the coastal region of Tanzania (Fig. 1). The environment in which groundwater sources are located represents the diversity of the local community in the Centre and peripheral areas of Bagamoyo to capture variations in groundwater source contamination risks. The groundwater sources in the study area consisted of boreholes, dug wells, and tube wells with hand pumps.

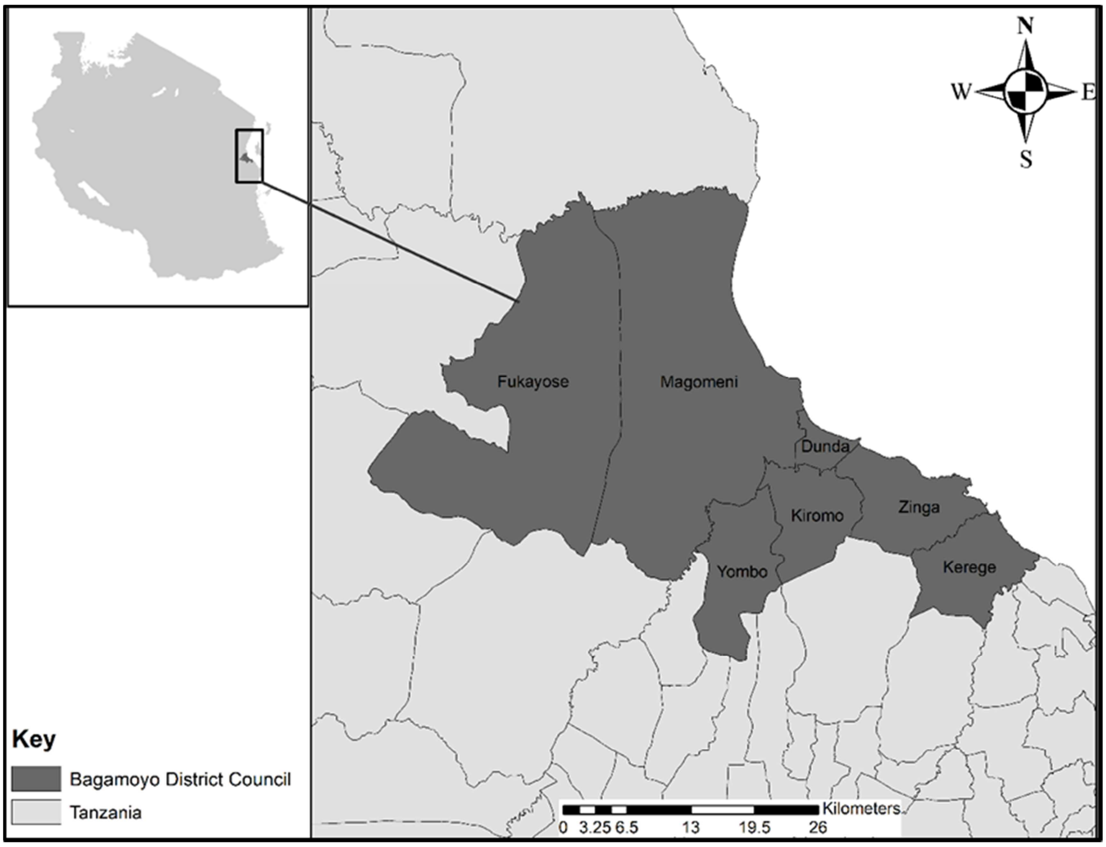

Fig. 1. Location of Bagamoyo district in Tanzania

Study design

A cross-sectional study was conducted to determine the prevalence of antibiotic-resistant *E. coli* in groundwater sources and factors associated with contamination between April to June 2023 in Bagamoyo district, Tanzania. Sanitary inspection, laboratory work on *E. coli* culture and disk diffusional methods were used to obtain the information required to attain the goal of this study. Subsurface and found in rocks and soil was defined as groundwater (springs and wells) (Indika *et al*., 2022). The groundwater sources actively in use were enrolled in this study to analyze the antibiotic susceptibility of all *E. coli* isolated from the sample collected.

Sampling collection

A total of 163 samples were collected from randomly picked groundwater sources in the study area. Sterile bottles (capacity of 125-ml) were used to collect samples from groundwater sources with care to avoid contamination of the samples (Kempthorne, 2006). Sampling steps were different depending on the type of facility. The hand pump was used to flush the tube well by pumping water for one minute. Afterwards, the bottle was opened, ensuring that only the outer edge of the cap was held and was filled to 125-ml line. The same sampling procedure was used to sample the borehole to the near tap connected to the pump.

A water sample from a dug well was collected using a 25-meter-long string and a cup. The cup slowly lowered into the well without touching the sidewalls or bottom of the well. The cup was raised, and water was filled into a sample collection bottle. All samples were stored in a cooler box with an ice pack, waiting to be delivered to Ifakara Health Institute Bagamoyo laboratory within six hours after sample collection.

Groundwater sanitary inspection

A sanitary risk assessment survey was undertaken on 163 groundwater sources in the study area to identify potential sources of microbial contamination and establish their contamination risk levels. The inspection approach, derived from WHO groundwater sanitary inspection guidelines (WHO, 1997), employs a checklist of contamination risk factors associated with on-site sanitation that affect groundwater. The procedure entailed physically assessing the groundwater sources, inspecting the surrounding environment, and noting the status (Yes or No) for each detected risk factor. The level of protection, closeness and position relative to uphill or downhill pit latrines were all taken into consideration. A "Yes" response indicated the existence of a microbiological contamination risk factor, whereas a "No" response showed its absence. The total number of existed risk factors was used to determine the sanitary score of each groundwater source. Finally, sanitary scores were categorized to obtain contamination risk levels of groundwater sources. Contamination risk levels were divided into four risk levels: very high (9-11), high (6-8), intermediate (3-5), and low (0-2).

Culture, isolation and identification of E. coli from the sample

Aqua Safe membrane filtration units were employed to filter 100 ml water samples using sterile membrane filters (MicroLab Scientific Gridded MCE) with a 47 mm diameter and 0.45µm pore size. Subsequently, these membrane filters were positioned straight onto Chromogenic Coliform Agar (CHROMagarTM CCA) plates and incubated (Heracell™ 150i CO_2 Incubator) at 37 °C for 24 hours. The appearance of metallic blue to violet colonies was taken as *E. coli* according to manufacturer's instruction manual. As a reference, the *E. coli* strain ATCC 25922 was used as control during the process.

Antibiotic susceptibility test of E. coli isolates

The disc diffusion method (CLSI, 2023) was employed to assess the susceptibility of *E. coli* isolates to twelve antibiotics. Isolates cultured in Chromogenic Coliform Agar were suspended in 0.9%w/v-NaCl (normal saline) using sterile plastic loop to attain turbidness of 0.5 McFarland standard. Sterile cotton swab was dipped into the inocula and streak the surface of Muller Hinton II agar (MHA) petri dishes. Antibiotics that were tested against *E. coli* isolates were: Amoxicillin-clavulanic acid 30µg, Ampicillin-sulbactam 20µg, Trimethoprim-sulfamethoxazole 30µg, Gentamicin 10µg, Ciprofloxacin 5µg, Chloramphenicol 30µg, Nalidixic acid 30µg, Nitrofurantoin 300µg, Cefazolin 30µg, Cefuroxime 30µg, Ceftriaxone 30µg, and Cefepime 30µg. antibiotics disk were placed onto the plates with forceps careful. Two plates with six discs each were incubated (Heracell™ 150i CO_2 Incubator) for 18- 24 hours at 37°C. Following CLSI guidelines, the zone diameter was used to interpret whether *E. coli* isolates were susceptible or not.

Multidrug resistance in E. coli isolates

The multidrug resistance was defined as resistance of *E. coli* isolates to at least one Cephalosporin antibiotic (Cefazolin, Cefuroxime, Ceftriaxone, and Cefepime) jointly with at least one of the remaining antibiotics tested.

Multiple antibiotic resistance index

The multiple antibiotic resistance index was calculated by dividing the number of antibiotics *E. coli* isolates were resistant to by the total number of antibiotics tested (Krumperman, 1983). A MAR index value greater than 0.2 suggests a greater risk of groundwater contamination in areas where antibiotics are often used (Davis and Brown, 2016). For this study, the MAR index was used to suggest exposure of groundwater sources to areas with high consumption of antibiotics and risk of resistant *E. coli* occurrence in groundwater facilities.

Data management and analysis

Data were gathered daily through the utilization of Google Forms and stored in Google Drive. Weekly, laboratory results were entered into Microsoft Excel Spreadsheet. Data was then exported to a Microsoft Excel Spreadsheet and integrated with the antibiotic resistance analysis. To ensure correctness, the data underwent reorganization and rigorous examination to find duplicates and structural problems. STATA 17 (StataCorp 4905 Lakeway Drive College Station, Texas 77845 USA) was employed for the comprehensive analysis of the data. Descriptive statistical analysis techniques were used to depict the prevalence of antibiotic resistance in *E. coli* regarding the tested antibiotics, as well as to evaluate multiple antibiotic resistance indices of *E. coli* isolates and the frequency distribution of sanitary risk factors and contamination risk categories of groundwater sources. The chi-square test was used to suggests significant different of antibiotic resistance.

Results

Sanitary inspection of the groundwater sources

For the 39 dug wells assessed, 89.7% (n =35) seem to have a poor seal-to-wall lining that allows water to enter the wells and 15.4% (n = 6) were located near a faulty or broken drainage channel permitting ponding. The study revealed that 65.6% (n = 42) of tube wells with hand pumps were located in areas with poor drainage causing stagnant water within 2m and 6.3% (n = 4) were located to other sources of pollution within 10m from tube wells. Moreover, 45% (n = 27) of borehole pump houses were within 15 to 20m from a latrine and none of the uncapped wells were observed within 15 to 20m of boreholes. Other sanitary factors observed around groundwater are described in Table 1 and 2.

Table 1. Potential sanitary risk factors of tube wells with hand-pump and dug wells

Sanitary factor	Dug well n (%)	Tube well/hand pump n (%)
Rope/hand pump/bucket at risk of contamination	34 (87.2)	35 (54.7)
Cracks in the concrete floor	26 (66.7)	24 (37.5)
Inadequate wall lining seal	35 (89.7)	-
Concrete floor < 1m	22 (56.4)	13 (20.3)
Inadequate parapet wall	22 (56.4)	9 (14.1)
faulty/broken drainage channel	6 (15.4)	7 (10.9)
Poor drainage within 2m	22 (56.4)	42 (65.6)
sources of pollution within 10m	12 (30.8)	4 (6.3)
The nearest latrine uphill	10 (25.6)	12 (18.8)
Latrine within 10m	11 (28.2)	13 (20.3)

Table 2. Potential sanitary risk factors of borehole

Sanitary factors	Number	Percentage (%)
A latrine within 15–20m	27	45.0
uncapped well within 15–20m	-	-
Damaged fencing	3	5.0
Permeable pump house floor	7	11. 7
Unsanitary sealed	15	25.0
Faulty/broken drainage	7	11. 7
Nearest percolating pit latrine	18	30.0
Other sources of pollution within 10m	5	8.3

Groundwater sources contamination risk level

The categorization of groundwater sources according to contamination risk levels revealed notable variations across different source types. Among the boreholes accessed in the study area, none were classified as having a "Very high" or "High" contamination risk. Instead, all boreholes were categorized as having a "Low" contamination risk level, which accounted for 73.3% (n=44) of the total boreholes surveyed. For tube wells with hand pumps, 32.8% (n=21) were classified as having either "High" or "Low" contamination risk levels, while only 6.3% (n=4) of the tube wells were categorized as having a "Very high" contamination risk. In contrast, a higher proportion of dug wells were found to be at greater risk of contamination. Specifically, 46.2% (n=18) of dug wells were classified as having a "High" contamination risk, while only 5.1% (n=2) were categorized as having a "Low" contamination risk. The detailed distribution of contamination risk levels by groundwater source facility is presented in Table 3.

Table 3. Frequency distribution of groundwater source facilities across contamination risk categories

Contamination risk categories	Boreholes n (%)	Tube well/hand pump n (%)	Dug well n (%)
Very high	-	4 (6.3)	4 (10.3)
High	-	21 (32.8)	18 (46.2)
Intermediate	16 (26.7)	18 (28.1)	15 (38.5)
Low	44 (73.3)	21 (32.8)	2 (5.13)

Table 4. Multidrug-resistant *E. coli* isolates in borehole, tube well and dug well

Variables	Total	Borehole n (%)	Tube well/hand pump n (%)	Dug well n (%)
No. of samples collected	163	60(36.8)	64 (39.3)	39 (23.9)
No. of samples with *E. coli* isolates	73(44.8)	31 (52)	15 (23)	27 (69)
No. of samples with resistant *E. coli* isolates	61(83.6)	25 (81)	12 (80)	24 (89)
Multidrug-resistant *E. coli* isolates	51(70)	23 (74)	8 (53)	20 (74)

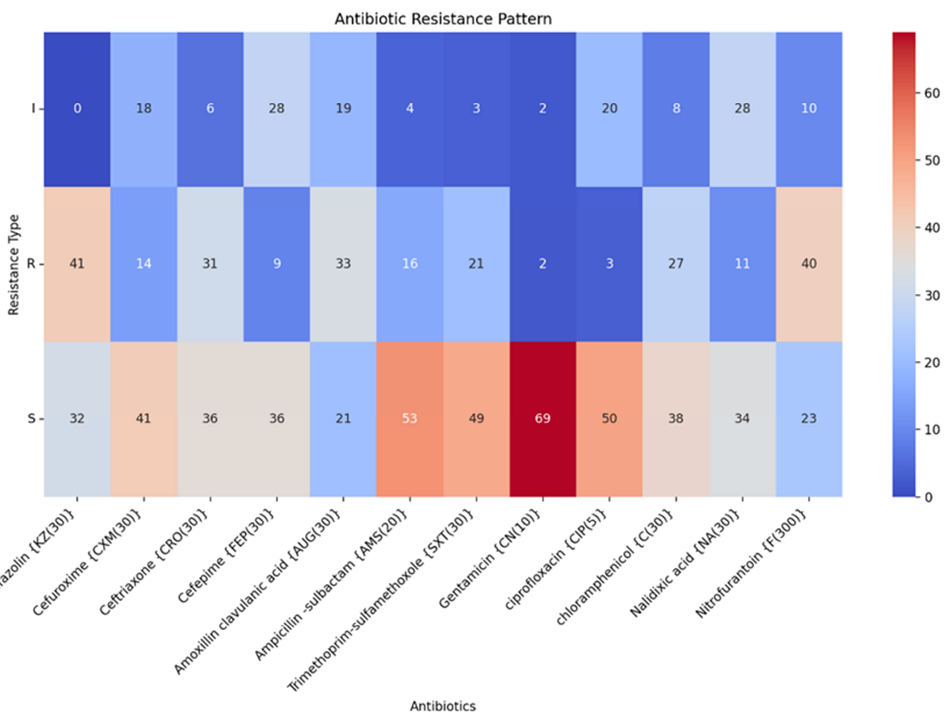

Fig. 2. The resistance pattern for each antibiotic heatmap, which shows the frequency of resistant (R), susceptible (S), and intermediate (I)

Antibiotic resistance of E. coli isolates

The results from 163 groundwater samples analyzed to determine the occurrence of *E. coli*, 44.8% (n = 73) tested positive for *E. coli*. The identified isolates were subjected to antibiotic susceptibility test, for the 73 of the bacteria, 83.6% (n = 61) showed resistance to one or more antibiotics, and 70% (n = 51) exhibited multidrug resistance. Notably, in the 39 dug wells, 89% (n = 24) revealed high rate of resistant *E. coli*. While multidrug-resistant *E. coli* was mostly detected from boreholes and dug well samples as shown in Table 4.

Profile of Antibiotic resistance in E. coli isolates

The Chi-square test for resistance distribution revealed a statistically significant difference in the proportions of resistance across the tested antibiotics (p-value < 0.001). The highest resistance rates were observed for Cefazolin (56.16%, n =41), Nitrofurantoin (54.79%, n=40), and Amoxicillin-clavulanic acid (45.21%, n=33). The lowest resistance rates were observed for Gentamicin (2.74%, n=2) and Ciprofloxacin (4.11%, n=3). These results highlight substantial variability in antibiotic resistance, with certain antibiotic exhibiting markedly levels of resistance than others as shown in Fig. 2.

Multiple antibiotic resistance index

The Multiple Antibiotic Resistance (MAR) index is a method for determining whether identified isolates originate from areas with high or low antibiotic usage. A MAR index exceeding 0.2 suggests a sensitive likelihood of contamination from sources where antibiotics are commonly employed. *Escherichia coli* isolates from the Bagamoyo groundwater source showed a MAR index of 0.28 whereas, the MAR index for isolates from dug wells range from 0 to 0.75. Isolates from boreholes and from tube wells with hand pumps had a MAR index ranging from 0 to 0.58.

Table 5. Frequency of groundwater sources contaminated with resistant *E. coli* in contamination risk categories

Contamination risk categories	No. of sample	No. of sample with *E. coli* isolates	Resistant *E. coli* isolates
Very high	8	3 (37.5)	3(100)
High	39	18 (46.2)	17 (94.4)
Intermediate	49	25 (52.0)	20 (80)
Low	67	27 (40.3)	21 (77.8)

Distribution of resistant E. coli across diverse contamination risk levels

The study found that a significant proportion of *Escherichia coli* isolates from groundwater were resistant to one or more antibiotics. Overall, 83.6% of all *E. coli* isolates exhibited antibiotic resistance. However, the prevalence of antibiotic-resistant isolates varied across different contamination risk categories. Specifically, all isolates (100%) from groundwater sources identified as being at very high risk of contamination were resistant to the tested antibiotics. In contrast, 77.8% (n=21) of *E. coli* isolates from sources with low risk of contamination showed resistance. These findings are summarized in Table 5, which details the distribution of resistant *E. coli* isolates across different contamination risk categories.

Discussion

The study's findings highlight significant public health concerns associated with groundwater contamination risks and the prevalence of antibiotic-resistant *E. coli* in groundwater sources. These findings reveal how environmental and sanitary factors around water sources influence the risk of microbial contamination, particularly for dug wells, tube wells, and boreholes.

The sanitary inspection findings revealed critical vulnerabilities in the groundwater source infrastructure, contributing to contamination risks. A significant proportion of dug wells exhibited inadequate wall sealing and were located near defective drainage channels, allowing water entry and potential contamination. The high percentage of poorly sealed dug wells indicates a substantial risk for contaminant intrusion, especially in areas prone to flooding or high surface water runoff. The most area depends in on-site sanitation has higher chance to groundwater contamination (Abanyie *et al.*, 2022). Hence, risk factors near groundwater sources suggest that the

Bagamoyo area relies heavily on on-site sanitation and highlighting the contamination dangers.

On other hand, the incidences of *E. coli* contamination are increasing due to consumption of water from contaminated water sources (Kumar *et al.*, 2020; Ngowi, 2020). This contamination led to spread of waterborne diseases that have been associated with consumption of contaminated drinking water (Hafiane *et al.*, 2020). This is the same as findings of this study in Bagamoyo where a considerable proportion of groundwater samples tested positive for *E. coli*, suggesting significant faecal contamination. Among positive samples, most of isolates displayed resistance to one or more antibiotic. The highest prevalence of antibiotic-resistant *E. coli* was observed in dug well samples that indicates their high exposure to environmental contamination sources. This might be due to the sanitary characteristics of the Bagamoyo town and how land use impacts the quality of groundwater sources (Da Silva Peixoto *et al.*, 2020). These findings suggest a strong link between inadequate water source protection and the persistence of antibiotic-resistant bacteria.

The antibiotic resistance profile of the isolates revealed that resistance rates varied widely among antibiotics. The presence of high resistance to commonly used antibiotics such as Cefazolin and Amoxicillin - clavulanic acid could be attributed to the extensive use of these antibiotics in human and veterinary medicine, which may drive resistance development (Katyali *et al.*, 2023; Salam *et al.*, 2023; Van *et al.*, 2020). This is proved by the MAR index observed in this study that exceed threshold of 0.2 which indicates that contamination may originate from areas with significant antibiotic use. This also reported to several of studies used MAR – index to predict antibiotic consumption similar to this study (Mir *et al.*, 2022; Sandhu *et al.*, 2016; Tahri *et al.*, 2021). The low resistance rates for Gentamicin and Ciprofloxacin could indicate that these antibiotics are either less frequently used in the study area or that resistance mechanisms to these drugs have not yet become widespread.

The prevalence of antibiotic-resistant *E. coli* provides critical insight into the impact of groundwater source hygiene on resistance patterns. All *E. coli* isolates from sources with "Very high" contamination risk demonstrated antibiotic resistance, suggesting that highly contaminated environments create conditions conducive to antibiotic-resistant bacteria proliferation (Viban *et al.*, 2021). Interestingly, even low-risk sources had a substantial percentage of antibiotic-resistant isolates, pointing to a pervasive issue with antibiotic resistance in the Bagamoyo's groundwater. This observation aligns with fact that not always sanitary risk score predict the groundwater contamination (Luby *et al.*, 2008). These findings indicate that groundwater sources in Bagamoyo may be subjected to contamination from antibiotic-laden sources, possibly through runoff or soil leaching.

Conclusion

The study highlights the urgency of improving sanitary protections around groundwater sources to mitigate contamination risks and the spread of antibiotic-resistant bacteria. Structural enhancements, such as better sealing and improved drainage systems, are essential to prevent surface water infiltration and contamination. The high prevalence of antibiotic-resistant *E. coli* isolates in groundwater indicates that antibiotic use and disposal practices in the region could be contributing to the resistance burden. These findings stress the need for comprehensive interventions to control environmental contamination and encourage the judicious use of antibiotics in the community. Furthermore, policies aimed at monitoring antibiotic resistance in groundwater sources should be implemented, alongside regular sanitary inspections and public awareness campaigns. Such measures are essential to protect public health, especially in communities that rely on groundwater as a primary drinking water source.

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