PREVALENCE OF ANTIBIOTIC-RESISTANT *ESCHERICHIA COLI* FROM GROUNDWATER SOURCES AND SANITARY INSPECTION FOR CONTAMINATION RISK IN BAGAMOYO DISTRICT, TANZANIA

Joram Japhet Mduda

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Public Health Research of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

July, 2024

ABSTRACT

In Low and Middle-income Countries (LMICs), people have access to water but have no access to safe, clean, and protected water sources. Globally, two billion people consume water contaminated with faeces, with *Escherichia coli* being the most prevalent pathogen in polluted water, posing significant health risks. This underscores the urgent need to investigate resistant *E. coli* from groundwater sources and to conduct sanitary inspections. In the Bagamoyo District, a cross-sectional study was undertaken, examining 60 boreholes, 39 dug wells and 64 tube wells with hand-pumps. *Escherichia coli* was isolated through a membrane-filtration method, and the disc diffusion method was used in susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) standards. The study found *E. coli* contaminated 44.8% of groundwater samples and 83.6% of *E. coli* isolates were resistant to at least one antibiotic. Resistance was highly observed to cefazolin (56.16%) and nitrofurantoin (54.79%), Moreover, 70% of the *E. coli* isolates were multidrug-resistant, with an overall MAR-index of 0.28. Sanitary inspections revealed that dug-wells (46.2%) were at high contamination risk, and tube wells with hand pumps (32.8%) were at high and low contamination risk levels. Also, boreholes (73.3%) were at low contamination risk. High *E. coli* contamination was observed in boreholes despite of being at low contamination risk. Furthermore, groundwater sources across contamination risk categories indicated high-rate-resistant *E. coli* contamination. Therefore, these findings highlight the presence of resistant *E. coli* from groundwater in Bagamoyo district and emphasize the necessity to promote proper antibiotic use to prevent contamination by resistant bacteria.

DECLARATION

I, Joram Japhet Mduda do hereby declare to the senate of Nelson Mandela African institution of science and technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institute.

Joram Japhet Mduda		17th July 2025
Candidate name	**Signature**	**Date**

The declaration is confirmed by:

Dr. Esther Gwae Kimaro		17th July 2025
Supervisor 1	**Signature**	**Date**
Dr. Catherine G. Mkindi		16th July 2025
Supervisor 2	**Signature**	**Date**
Dr. Anita Rugaika	ARugaika	16th July 2025
Supervisor 3	**Signature**	**Date**

COPYRIGHT

This dissertation is copyright material protected under the Berne Convention, the Copyright Act of 1999, and other international and national enactments, on that behalf, of intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review, or discourse with an acknowledgment, without the written permission of the office of Deputy Vice-Chancellor for Academic, Research, and Innovation on behalf of both the author and the Nelson Mandela African Institute of Science and Technology.

CERTIFICATION

The cosignatories certify that they have revised the dissertation titled "Prevalence of antibiotic-resistant *Escherichia coli* from groundwater sources and factors associated with contamination in Bagamoyo district, Tanzania" and endorsed for examination in partial fulfillment of the requirements for the degree of Master in Public Health Research of the Nelson Mandela African Institution of Science and Technology.

Dr. Esther Gwae Kimaro

Supervisor 1 **Signature** **Date**

Dr. Catherine G. Mkindi

Supervisor 2 **Signature** **Date**

Dr. Anita Rugaika

Supervisor 3 **Signature** **Date**

ACKNOWLEDGEMENTS

First and foremost, I want to express my gratitude to God, my living Savior, for his graciousness and mercy in allowing me to study at the Nelson Mandela African Institution of Science and Technology in safety while also providing me with good security and psychological fitness.

I want to express my sincere gratitude to my supervisors, Dr Catherine G. Mkindi of the Ifakara Health Institute, Dr. Esther G. Kimaro, and Dr Anita Rugaika of the Nelson Mandela African Institution of Science and Technology, for their diligent oversight and constructive criticism that helped to ensure the successful completion of the research and the writing of this dissertation, Tarsis Mlaganile and Teckla Kazimoto of IHI for their assistance in laboratory and field works. Their contributions are much valued. Furthermore, I appreciate the tolerance of my lovely family during the time of my studying and research.

DEDICATION

This section of my work is dedicated to my devoted wife Grace Richard Shani and my daughters Giana & Joana Joram Mduda. I was able to get to this point because of their motivation and inspiration for the whole period of my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABR	Antibiotic Resistance
AMR	Antimicrobial Resistance
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
IHI-IRB	Ifakara Health Institute Institutional Review Board
MAR	Multiple Antibiotic Resistance
NM-AIST	Nelson Mandela African Institution of Science and Technology
OR	Odd Ratio
UNGA	United Nation General Assembly
UNICEF	United Nations International Children Emergency Fund
URT	United Republic of Tanzania
UTI	Urinary Tract Infection
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Worldwide, 2 billion individuals are estimated that are using faecal polluted water, primarily in developing nations (WHO/UNICEF Joint Monitoring Programme & for Water Supply, 2020). The fact that individuals are consuming tainted water that contains bacteria like *Escherichia coli* (*E. coli*) is the most concerning worry on public health (Nowicki *et al.*, 2021; Odonkor & Ampofo, 2013). According to WHO (2019), *E. coli* is among the bacteria that causes epidemics of diarrheal illness in human and is accounted for 370 000 child deaths annually globally. Moreover, *E. coli* causes urinary bacteremia, pneumonia, UTIs, and peritonitis (Jain *et al.*, 2015; Nielsen *et al.*, 2014).

Escherichia coli is one of the ten public health hazards coming from the misuse of antibiotics for human and animal therapies, food production, and environmental disposal. Despite of causing infections, *E. coli* has been related to the growing global problem of antimicrobial resistance (WHO, 2021b). Antimicrobial resistance happens when micro-organisms such as viruses, fungi bacteria, and parasites evolve and become less responsive to antimicrobial treatments. This increases the difficulty of treating infections and lowers the chance of managing serious illness that leads to, death, and the spread of disease (WHO, 2021a). If the current trend continues, resistant infections are estimated to cause 4.2 million individual deaths in Africa per year by the year 2050, with Sub-Saharan Africa accounting for the majority of these deaths (Neill, 2016a). Research conducted in Morocco and northern Tanzania, along with a global scoping review by Chique *et al.* (2021), has linked antimicrobial resistance risks to water sources. These studies informed an increase of resistant *E. coli* in water sources across several African countries (Lyimo *et al.*, 2016; Tahri *et al.*, 2021).

Three main processes underlie resistance to antibiotics: (1) breakdown of antibiotic drugs by enzymes; (2) alteration of bacterial proteins targeted by antibiotics; and (3) modification of membrane penetrability to antibiotics (Dever & Dermody, 1991; Reygaert, 2016). It has also been reported that *E. coli* can become resistant to beta-lactam antibiotics over the action of extended-spectrum beta-lactamase (ESBL), a plasmid-mediated enzyme that hydrolyze and deactivate beta-lactams like cephalosporins and monobactams (Chmielarczyk *et al.*, 2014).

Gram-negative bacteria such as *E. coli* employ efflux pumps or alter membrane permeability to minimize the concentration of the drug within the cell. Additionally, they utilize various enzymatic mechanisms to enhance the binding of antibiotics to their target sites, thereby inactivating the antibiotics (Arbab *et al.*, 2021; Meletis & Bagkeri, 2013).

Numerous researches carried out in Tanzania about multi-drug resistance in water sources discovered an increase of resistant microbes, with *E. coli* being among of the bacteria exhibiting multi-drug resistance in urban water sources (Ripanda *et al.*, 2023a; Subbiah *et al.*, 2020a). Research carried out in Tanzania has demonstrated the existence of resistant *Escherichia coli* in freshwater, marine, rodent, chicken, domestic soil, and meat products (Marijani, 2022; Sonola *et al.*, 2021). This finding appears to indicate a potential risk of exposure to groundwater sources. Since Tanzania has prioritized multisector surveillance on antimicrobial resistance in humans, animals, plants, and the environment in its action plan (URT, 2017), knowing the status of *E. coli* antibiotic susceptibility in groundwater sources is important not only for the implications for healthcare that arise from having limited treatment options but also for the financial implications of using expensive treatments and the length of time that the infected population remains ill.

It is acknowledged that groundwater contamination poses a serious risk to public health, particularly in boreholes, tube wells, and excavated wells (Bivins *et al.*, 2020; Viban *et al.*, 2021). Pit latrines, pour-flush toilets that empty into a pit or septic tank, and even open defecation are common in many developing nations (Ercumen *et al.*, 2017), especially in impoverished neighborhoods. Seasonal flooding, poor sewage systems, high population densities, improper disposal of industrial waste, poor hygiene practices, and broken septic systems all contribute to this problem systems (Da Silva Peixoto *et al.*, 2020; Ferrer *et al.*, 2020). All of these elements together increase the possibility of contaminating sources of drinking water. Furthermore, according to Kamara *et al.* (2022), *E. coli* is a conventional bacterial biomarker of faecal pollution in groundwater, even though the World Health Organization recommends that there should be no trace of *E. coli* in hundred milliliters of drinking water.

The majority of the settlements in Bagamoyo, which is located on Tanzania's coast, rely on groundwater as a backup supply because of the inconsistent supply of piped water (Ligate *et al.*, 2021). Bagamoyo district being in the coastal area groundwater contamination may be attributed to shallow water table (Nkotagu, 1989). According to Amos *et al.* (2014) and Ramatla *et al.* (2023), this encourages *E. coli* to spread to a bigger population. This emphasizes the necessity for the survey in the Bagamoyo area to look into the status of the resistant *E. coli*, which is primarily known to cause pollution in groundwater sources elsewhere.

1.2 Statement of the problem

Access to safe, clean and affordable water for consumption is an important human right, as recognized by Sustainable Development Goal 6, aiming to achieve global delivery of safe water (Ercumen *et al.*, 2017). Safe water, devoid of microbial contamination, is crucial for various domestic, recreational, and drinking purposes without compromising human health (WHO, 2023). However, in the Bagamoyo district, despite the availability of treated municipal tap water, residents predominantly rely on a diverse array of water sources, including municipal taps dispensing treated river water, shallow wells, rain harvesting, and boreholes (Mattioli *et al.*, 2014). This reliance on groundwater sources is often due to intermittent and unreliable water supply from the municipal system, leading people to store water in cisterns or tanks and resort to groundwater alternatives (Rubino *et al.*, 2019).

In Bagamoyo District is covered by the municipal water supply and groundwater remains a crucial supplementary source. However, studies indicate that groundwater sources, particularly shallow wells and boreholes, along with stored water, are vulnerable to contamination by pathogenic *E. coli* (Mattioli *et al.*, 2014), some of which may exhibit resistance to antibiotics. Research conducted in Bagamoyo has reported a high prevalence of pathogenic *E. coli* in unimproved water sources than in improved ones, highlighting the potential health risks related to untreated water consumption (Mattioli *et al.*, 2013).

Previous studies indicate *E. coli* serves as an important contributor to the spread of resistance genes, posing additional challenges to public health (Katakweba *et al.*, 2018). Despite the consensus on the existence of *E. coli* in groundwater, there remains a lack of comprehensive research on its antibiotic sensitivity, thereby raising concerns about the potential implications for human health. As people consume untreated groundwater contaminated with antibiotic-resistant *E. coli* jeopardizes human health, contributing to inadequate prevention and control of infection and facilitating the spread of antibiotic resistance (Odonkor & Addo, 2018). Therefore, it is urgently needed to assess the prevalence of resistance in *E. coli* in groundwater, understand its spatial distribution, and conduct sanitary inspections to identify risk of contamination of groundwater in the Bagamoyo district.

1.3 Rationale of study

Enhancing comprehension of the antibiotic resistance dynamics in isolated *E. coli* from groundwater sources, along with identifying associated contamination factors, is vital for effectively recognizing and mitigating resistant microbials outbreaks within community settings. The existence of resistant *E. coli* in groundwater not only poses immediate health risks but also contributes to the broader issue of resistance, undermining infection control efforts and treatment. Despite the critical nature of this issue, there is a notable gap in research regarding the resistance status of *E. coli* from groundwater sources in the Bagamoyo district council, Tanzania. This study intends to assess the prevalence and spatial distribution of antibiotic-resistant *E. coli* in groundwater sources, thereby providing essential data to inform public health control strategies. Also, conducting sanitary inspections, the study will also identify contamination sources and guide targeted interventions to reduce public health risks. Ultimately, this research will support the objectives of Sustainable Development Goal number 6 by promoting the delivery of safe and affordable drinking water, thus enhancing public health and well-being in the Bagamoyo district.

1.4 Study objectives

1.4.1 General objective

To determine the prevalence of resistant *E. coli* from the groundwater and their sanitary contamination risk in the Bagamoyo district council

1.4.2 Specific objectives

(i) To determine the prevalence of resistant *E. coli* in groundwater sources

(ii) To determine spatial distribution of groundwater facilities contaminated with resistant *E. coli*

(iii) To determine the contamination risk levels of groundwater sources

1.5 Research questions

(i) What is the prevalence and antimicrobial resistance profile of *Escherichia coli* isolated from groundwater sources in Bagamoyo District?

(ii) How are the groundwater sources contaminated with antibiotic-resistant *E. coli* spatially distributed across Bagamoyo District?

(iii) What sanitary risk factors and contamination risk levels of groundwater?

1.6 Significance of study

This study provides substantial insights into resistance within *E. coli* populations sourced from groundwater in Bagamoyo, Tanzania. Furthermore, it elucidates the sanitary factors linked to concerns regarding groundwater contamination attributable to antibiotic-resistant *E. coli* strains. Identifying the presence and spatial dispersion of resistant *E. coli* is envisioned to contribute to devising preventive measures against potential infections. This holds particular significance, given that inadequately treated infections may lead to prolonged illness or even fatalities. Additionally, grasping the contamination risk level enables the implementation of targeted interventions addressing risk factors specific to distinct locations within groundwater sources.

1.7 Delineation of the study

Assessing the prevalence of antibiotic resistance of *E. coli* in groundwater and their sanitary inspection to determine contamination risk is crucial to protecting public health from consuming contaminated groundwater. The research focused on various groundwater sources utilized by Bagamoyo district residents, including dug wells, boreholes, and tube wells with hand pumps. Given the intermittent and unreliable municipal water supply, many residents rely on groundwater as alternatives, which are vulnerable to *E. coli* contamination. This study employed a cross-sectional design to collect water samples from different sources and locations, ensuring representative sampling across the district. Laboratory analyses were conducted to detect *E. coli* and determine its antibiotic resistance profiles. Google Earth tools used to map the distribution of contaminated sources, and sanitary inspections are carried out to identify factors contributing to contamination and the contamination risk level. The findings provide critical insights into the prevalence of resistant *E. coli,* its spatial distribution, and the associated health risks in the district, thereby informing public health interventions and policy decisions aimed at improving water quality and managing antibiotic resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background of *E. Coli*

Escherichia coli, as part of the fecal coliform family, is commonly found in various environments, including food, water, and in the animals' and human intestines. While many strains of *E. coli* do not cause illness, certain strains lead to diarrhea. Dr. Theodore Esherich, a German scientist in the late 19th century, first identified *E. coli*, which subsequently became a model bacterium in molecular biology laboratories worldwide (Hacker & Blum-Oehler, 2007). One notable strain, Shiga toxin-producing *E. coli* O157:H7, was discovered in the USA in 1982 during an outbreak of bloody diarrhea (Law, 2000). This outbreak was connected to the ingesting of undercooked meat, raw milk, and contaminated water. Following this event, numerous studies have been undertaken globally to examine the prevalence of *E. coli* in various sources, including water, caregiver hands, stored water, meat, fish, milk, and human urine (Roberts *et al.*, 2022; Sonola *et al.*, 2022). The WHO has acknowledged *E. coli* O157:H7 as a significant cause of diarrhea outbreaks worldwide, particularly when contaminated water and food are consumed (Coleman *et al.*, 2012). This recognition underscores the importance of ongoing research and monitoring efforts to mitigate the risks associated with this pathogen.

2.2 Significance of antibiotics in public health

Since their detection in the early 20th century, antibiotics have significantly enhanced both public health and quality of life. They are considered miraculous medications that have saved countless lives (Muteeb *et al.*, 2023). However, widespread misapplication of antibiotics in human, and animal husbandry, and agriculture leads to the development of resistant bacteria (Manyi-Loh *et al.*, 2018; Salam *et al.*, 2023; Van *et al.*, 2020). The environment now contains more active antimicrobial agents due to overuse, which encourages resistant gene selection (Serwecińska, 2020). Groundwater is directly contaminated by wastewater and fecal effluents, which contributes to the development and dissemination of resistance in bacteria (Nnadozie & Odume, 2019). When bacteria resistant to antibiotics get into waterways, they can either infect people or animals through consumption, or they can spread their resistant genes to other bacteria in the surrounding environment (Sanderson *et al.*, 2018). Groundwater sources are crucial to the community's ability to receive a consistent supply of water in Bagamoyo.

However, because most developing nations have resistant bacteria, these water sources may be vulnerable to contamination from sanitary facility effluents (Aslan *et al.*, 2018; Bojar *et al.*, 2021; Praveenkumarreddy *et al.*, 2020). Antibiotic-resistant bacteria have raised concerns about the environment and water sources in Tanzanian cities and developing towns, and these concerns call for quick attention (Lyimo *et al.*, 2016; Subbiah *et al.*, 2020a). Nevertheless, there is insufficient proof of water source contamination from these regions, including the claimed Bagamoyo district.

2.3 Antibiotic-resistance mechanism

Bacteria employ three fundamental mechanisms to resist antibiotics: enzymatic degradation of antibiotic drugs, alteration of targeted bacterial proteins by antibiotics, and modification of membrane penetrability to antibiotics (Dever & Dermody, 1991; Reygaert, 2016) Extended-spectrum beta-lactamase (ESBL), a plasmid-mediated enzyme, is capable of hydrolyzing and deactivating beta-lactam antibiotics like cephalosporins and monobactams, contributing to Escherichia coli's resistance development (Chmielarczyk *et al.*, 2014). Antibiotic resistance can arise through enzymatic effects that hinder easy binding to target sites, achieved through the destruction or alteration of antibiotic structures, employing hydrolysis, group transfer, and redox reactions (Varela *et al.*, 2021). Additionally, bacteria utilize efflux pumps or modify outer membrane porins to restrict antibiotic entry into cells (Meletis & Bagkeri, 2013). Moreover, ESBL-producing *E. coli* can spread from person to person through physical contact and antibiotic overuse and misuse in agriculture exacerbating the global rise of antibiotic resistance (Mgaya *et al.*, 2021; Ventola, 2012). Consequently, antibiotic-resistant *E. coli* strains are increasingly prevalent worldwide, highlighting the urgency of addressing this global concern (Shlaes *et al.*, 2013).

2.4 The global problem of antibiotic resistance

Resistance to antibiotics has reached a tipping point and is now an international danger to public health (Mario Galindo-Méndez, 2020). According to Murray *et al.*, 2022 in 2019, 4.95 million deaths were related with antimicrobial resistance internationally, 1.27 million mortality were attributable to bacteria resistance and the lead bacteria is *E. coli*, especially in sub-Saharan Africa. Because of their high vulnerability to antibiotic resistance, *E. coli* is the most serious threat from antibiotic resistance. *E. coli* is particularly concerning in public health because they endanger patients and cause prolonged morbidity, meaning that antibiotic resistance exacerbates treatment problems, demanding measures (Azuma *et al.*, 2022). **Moreover, it** is estimated to cause 10 million mortalities in the next 35 years to come worldwide and most of the deaths will happen in low-income countries and sub-Saharan African countries (WHO, 2014). Which will cause 100 trillion USD of global production to be lost (O'Neill *et al.*, 2021, 2021). According to Founou *et al.* **(**2017) five out of the six WHO provinces had over 50% resistance to third-generation antibiotics in *E. coli*. Likewise, Tanzania was reported to be resistant to cephalosporin of the third generation (Moremi *et al.*, 2014). Similar antibiotic resistance of enteric pathogens was reported in studies in the southern coast region of Tanzania from chicken slaughter, wastewater, and water sources which are common transmission paths of enteric diseases (Lyimo *et al.*, 2016; Mgaya *et al.*, 2021; Ripanda *et al.*, 2023b).

2.5 A conceptual framework on *E. coli* contamination in groundwater and antimicrobial resistance

Groundwater, long regarded as a safe drinking water source, is increasingly vulnerable to contamination by *Escherichia coli* due to poor sanitation, animal waste, and environmental mismanagement (Ashbolt *et al.*, 2001). *E. coli* serves as a reliable fecal indicator, and its presence often signals the risk of enteric pathogens such as Salmonella and Shigella. Contamination pathways include surface runoff, seepage from unlined pit latrines, and infiltration from poorly managed wastewater (Howard *et al.*, 2003; Luby *et al.*, 2008a).

The growing challenge of antimicrobial resistance (AMR) adds further complexity. Resistant *E. coli* strains, originating from humans or animals treated with antibiotics, can enter the groundwater system and persist in the environment (Berendonk *et al.*, 2015). In low-resource settings such as Tanzania, misuse of antibiotics is widespread due to weak regulations, enhancing the selection pressure for resistance(Taneja & Sharma, 2019). These bacteria may share resistance genes through mechanisms such as horizontal gene transfer.

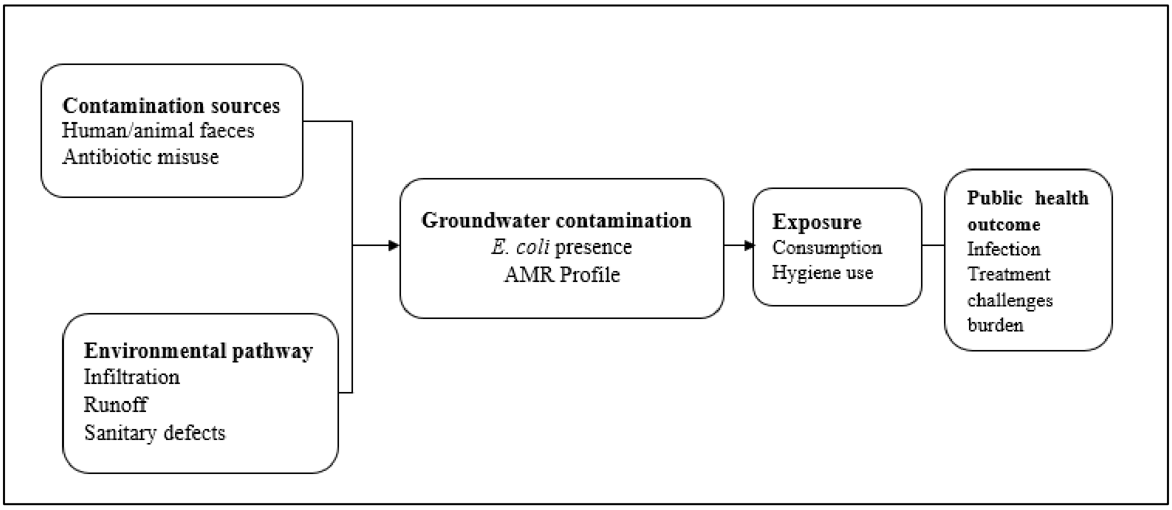

Figure 1: Conceptual framework on *E. coli* contamination and AMR

The conceptual framework follows a transmission chain: source (fecal waste with AMR *E. coli*) environmental media (soil, runoff, groundwater) exposure (drinking water, hygiene use). This process is influenced by land use, proximity of pollution sources to water points, rainfall patterns, and sanitation conditions. The presence of multi-drug-resistant strains even in low concentrations poses elevated health risks due to reduced treatment options.This framework emphasizes the interconnected nature of environmental and public health risks, supporting integrated approaches like One Health and Water Safety Plans to address both microbial contamination and AMR threats (WHO, 2011).

2.6 The burden on public health

The presence of *Escherichia coli* in drinking water, especially from untreated groundwater sources, significantly contributes to the global burden of waterborne diseases. According to the World Health Organization (WHO, 2022b), unsafe water, sanitation, and hygiene are responsible for over 1.7 billion cases of diarrhea annually, with the highest impact observed among children under five in Sub-Saharan Africa (Prüss-Ustün *et al.*, 2014). Ingestion of contaminated water can lead to various health conditions, including diarrhea, urinary tract infections, and life-threatening illnesses such as septicemia.

This public health threat intensifies when *E. coli* strains exhibit resistance to commonly used antibiotics. Antimicrobial resistance (AMR) leads to ineffective treatment, prolonged illness, higher medical expenses, and increased mortality (Neill, 2016b). In rural Tanzanian settings like Bagamoyo District, limited diagnostic capacity and restricted access to second-line antibiotics further compound the problem.

Moreover, AMR *E. coli* spreads silently through environmental exposure particularly through contaminated water bypassing traditional clinical surveillance systems. Asymptomatic carriers can unknowingly facilitate transmission within households and communities (Walsh, 2013). This environmental pathway is especially concerning in densely populated areas with inadequate sanitation and shared water sources.

Economically, the burden is widespread. At the household level, families face increased health costs and lost income. At the national level, the healthcare system is strained, and progress toward Sustainable Development Goal 6 (clean water and sanitation for all) is jeopardized.

In conclusion, the intersection of *E. coli* contamination and AMR presents a critical challenge to public health. Addressing it requires integrated strategies involving water quality monitoring, AMR surveillance, community education, and investment in WASH infrastructure.

2.7 Groundwater sources and their classification

Groundwater is a vital resource for drinking, agricultural, and industrial requirements in incomes countries and it is an essential part of the global water cycle (Carrard *et al.*, 2019). Comprehending the classification of underground water sources is essential for efficient administration and preservation endeavors. Geological formations, hydrological features (confined and unconfined aquifers, perched aquifers, and artesian wells), accessibility and construction (dug wells, boreholes, tube wells, and springs), and depth below the surface (from shallow to deep groundwater) are some of the factors that can be used to categorize groundwater (Adnan *et al.*, 2019; Balasubramanian *et al.*, 2015). This classification makes it easier to monitor and evaluate specific aspects of water quality, such as chemical composition, microbial contamination, and pollutant levels.

2.8 Vulnerability of groundwater contamination

Groundwater sources are susceptible to pollution from a variety of sources and their vulnerability is increased by being close to sanitation infrastructure, such as sewage treatment plants and septic tanks (Hynds *et al.*, 2012). Whereby, untreated sewage can introduce bacteria such as *E. coli* into groundwater from these contamination sources (Rao *et al.*, 2013). Abuse of antibiotics and inappropriate waste disposal are among the factors that contribute to the occurrence of resistant *E. coli*. However, agricultural, urbanization, and runoff contribute to groundwater contamination with resistant *E. coli* through porous formations and cracked bedrock that promote quick penetration of microbes if the source is not protected (Almakki *et al.*, 2019; Ibekwe *et al.*, 2011). The use of antibiotics in animals and poor waste management are two ways that agriculture contributes to groundwater contamination especially in area with careless oversight and restrictions (Manyi-Loh *et al.*, 2018). To address this, more stringent policies, sustainable agriculture, better sanitation, and ethical antibiotic usage are needed to protect public health by addressing the sources of contamination.

2.9 Sanitary inspection for groundwater sources

The World Health Organization (WHO) defines a sanitary inspection as an on-site assessment of a water supply aimed at identifying the sources of current and possible contamination (WHO, 1996). Sanitary inspection is further defined as an assessment of risk factors visually that could lead to fecal pollution of water sources (Kelly *et al.*, 2021; Pond *et al.*, 2020). It has been suggested that standardized sanitary inspection be used as a strategy to improve water quality at its sources and encourage the supply of safe water (WHO, 2022a). Throughout this procedure, forms that have been expressly created to enable a thorough risk-based evaluation of water systems are used and questions about possible risk issues surrounding the water supply are included in the sanitary inspection form (Odjegba *et al.*, 2020).

2.10 Contribution of sanitary factors to the occurrence of resistant *E. coli*

Groundwater sources have the potential of getting antibiotics contamination and with bacteria that are resistant, and genes that cause antibiotic resistance from a diversity of sources, including agriculture, and sanitation facilities, which play a critical role in the development and spread of resistance (Kusi *et al.,* 2022). It is estimated that 90% of consumed antibiotics are found in the urine and faeces of the patient (Singer *et al.,* 2016) that is disposed of in sanitary facilities. The dose of the antibiotic present in the environment is less compared to that intended to kill bacteria such as *E. coli,* this promotes the mechanism of bacteria to create resistance to antibiotics (Kraupner *et al.,* 2018). There is proof that environmental bacteria such as *E. coli* can spread easily to areas with poor sanitation services and unimproved water sources (Caudell *et al.*, 2018). Therefore, groundwater that is in a urine and feces-contaminated environment is at high risk of containing resistant *E. coli* (Leonard *et al.*, 2018).

The majority of enteric pathogen infections including *E. coli* are acquired through ingestion and spread from one host to another through several channels. Among those channels are improper toilet positioning and waste disposal that act as the center of enteric pathogens transmission between humans, humans and animals, humans, animals, and the environment (Subbiah *et al.*, 2020b). Even though access to safe drinking water, good sanitation, and hygiene education is a human right (UNGA, 2010), only 53.6% of Tanzanians had access to basic toilets, and 3% practiced open defecation (NESR, 2018). This acts as the center of transmission of resistant *E. coli* to the environment including groundwater sources and soil to many informal settlements and rural populations in developing nations due to the use of improper sanitation facilities and reliance on shared pit latrines and open defecation (Collignon *et al.*, 2018; Omulo *et al.*, 2021). In addition, it was reported that *E. coli* is prevalent in most of the privately owned water sources in developing nations (Genter *et al.*, 2021; Wang *et al.*, 2022). Therefore, assessing the susceptibility of *E. coli* isolated from groundwater is imperative in the process of controlling and preventing the spread of resistant bacterial infection.

2.11 Spatial distribution of *E. coli* contamination in groundwater

Groundwater containing resistant *E. coli* poses a serious threat to worldwide public health. Different regions have different prevalence and distribution of these resistant strains due to factors like population density, wastewater treatment, and agricultural methods. This literature review looks at the worldwide and Sub-Saharan African groundwater-resistant *E. coli's* spatial distribution.

Studies reveal that antibiotic-resistant *E. coli* is a common cause of groundwater contamination on all continents, with local behaviors and legislation influencing the extent of the contamination. American research, for instance, found antibiotic-resistant *E. coli* in 20% of groundwater samples close to cattle farms, indicating a considerable influence from agricultural runoff (Zhang *et al.*, 2020). Comparably, research conducted in Europe has shown that resistant *E. coli* may be found in groundwater in both rural (37.5%) and urban areas (16.7%) (AbuAlshaar *et al.*, 2022; Andrade *et al.*, 2022). This can be attributed to inadequate wastewater treatment and excessive use of antibiotics in agriculture (Lanyon *et al.*, 2021).

Studies indicate the main causes of contaminated groundwater are leakage from septic tanks, incorrect disposal of pharmaceutical waste, and agricultural runoff. The distribution of contaminants is significantly influenced by environmental conditions, including aquifer features, soil type, and rainfall patterns (Li *et al.*, 2021). The use of antibiotics on cattle and intensive farming methods are major factors in the proliferation of resistant bacteria in groundwater. Sewage leaks and industrial waste make the issue worse in urban areas and encourage resistance to spread (Ayad *et al.*, 2021).

Antibiotic-resistant *E. coli* is very common in groundwater throughout Sub-Saharan Africa, particularly in areas with poor access to clean water and sanitary facilities. Significant levels of resistant *E. coli* have been found in studies from Kenya, Nigeria, and South Africa, with notable differences between rural and urban locations. For example, studies conducted in Nairobi revealed that multi-drug resistance *E. coli* was present in 30% of groundwater samples from informal settlements (Ombuya *et al.*, 2023). Poor sanitation infrastructure and insufficient wastewater treatment facilities are two of Sub-Saharan Africa's biggest problems. Numerous populations rely on open defecation and pit latrines, which significantly contaminate groundwater (Vermeulen *et al.*, 2015). The problem is further made worse by the absence of strict laws governing the use of antibiotics in both humans and animals. Urban slums with high population densities, poor water and healthcare systems, and other factors provide breeding grounds for resistant microorganisms (Godman *et al.*, 2022). Groundwater contamination is also greatly exacerbated by agricultural practices, such as the irrigation of untreated effluent. To address these problems and protect public health, a multifaceted strategy including better cleanliness, stricter antibiotic use laws, and extensive monitoring is needed.

2.12 Importance of antibiotic resistance surveillance in groundwater sources

Antibiotic resistance investigation is serious for guiding therapeutic decisions about empirical treatment and tracking changes or variations in resistance over time and its distribution (Johnson, 2015). Microbiology laboratories play an important role in the generation of core surveillance data by consistently detecting and assessing the antibiotic susceptibility of bacteria isolated from various specimens.

Surveillance, as defined by the Centers for Disease Control and Prevention (CDC), involves the organized gathering, examination, and understanding of health data necessary for public health action (Thacker & Berkelman, 1988). Prevalence surveillance, in particular, captures data over a set length of time, allowing for regular temporal trend analysis and providing insights into resistance trends over extended periods (Johnson *et al.* 1996). This type of surveillance tracks disease resistance at local, regional, and national levels, providing valuable information to healthcare professionals, researchers, pharmaceutical businesses, and regulatory bodies. In hospitals, surveillance data is used to monitor local antibiograms, develop infection control strategies, and establish antibiotic usage guidelines (Masterton, 2000). Given this context, the availability of data on antibiotic resistance in groundwater sources within communities is crucial for monitoring public health hazards and the effects of prophylactic antibiotic use. Understanding the prevalence of *E. coli* in groundwater sources and its resistance profile in growing towns like Bagamoyo is vital. The current study aims to contribute to proper mitigation and preventive mechanisms against the rapid growth of antimicrobial resistance in Tanzania by investigating the resistance of *E. coli* in groundwater sources in Bagamoyo. This research will provide insights into the contamination risks and resistance trends in the area, aiding in the development of effective public health strategies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Pwani region in Tanzania, at the Bagamoyo District Council, which is situated at 6.4428° S and 38.9085° E (Fig. 1). The groundwater sources in the research area were hand-pumped tube wells, dug wells, and boreholes. Bagamoyo was selected due to its growing demand of water for various uses such as industrial, commercial and residential. Furthermore, many researches about the water sources contamination had been conducted in cities and highly populated region that led to lack of consideration in water source management in peri-urban area.

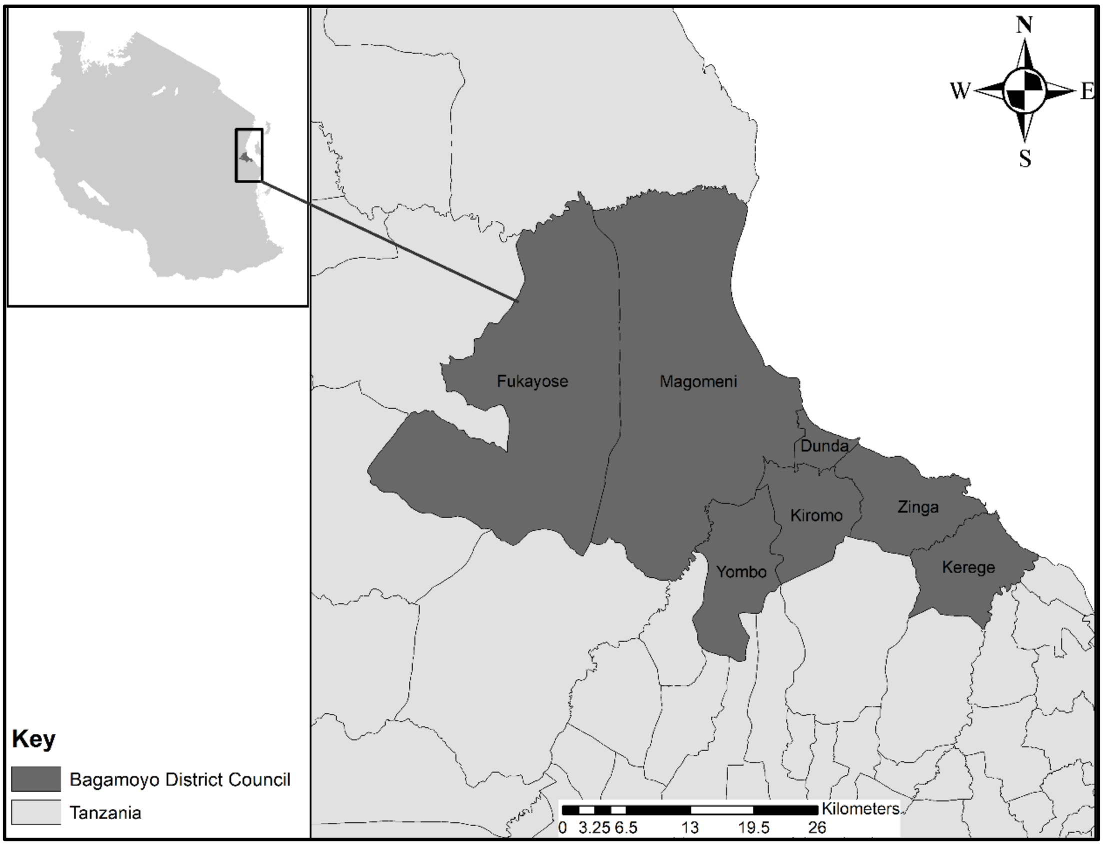

Figure 2: Map showing the location of Bagamoyo District Council

3.2 Study design

From April to June 2023, a cross-sectional study design was used to ascertain the prevalence of resistant *E. coli* in groundwater sources and parameters related to contamination. Disc diffusional procedures, sanitary inspection, and *E. coli* culture activities in the lab were utilized to gather the data needed to complete this study. Groundwater, which includes springs and wells, is described as the water that is found beneath the surface and in rocks and soil (Indika *et al.*, 2022). In order to examine the resistance of the *E. coli* isolates, the groundwater sources that are currently in use were included in this investigation.

3.3 Sample size calculation

With a power of 80% at a 95% confidence interval, a total of 163 water samples from 163 groundwater sources were collected in this study with a maximum margin error of 0.05. The samples size was obtained from the formula below (Okunade, 2018):

$$N = \frac{P(1-P)Z^2}{E^2}$$

Where: -

N= Estimated Number of water samples from groundwater sources

Z= Value based on 95% confidence level at (1.96)

P = Sample proportion (88%)

E = Margin of error (0.05)

From the formula above

$$\mathrm{N} = \frac{0.88(1-0.88)1.96^2}{0.05^2}$$

$$\frac{0.40567}{0.0025} = 162.3$$

Which were approximated to 163 samples

3.4 Criteria for sampling groundwater sources

3.4.1 Inclusion criteria

(i) Groundwater sources from boreholes, dug wells, tube well or springs

(ii) Groundwater sources being actively used

(iii) Groundwater sources within five selected wards of Bagamoyo district

3.4.2 Exclusion criteria

(i) Groundwater sources used for fish rearing or harvested to be used in rearing fish

3.3 Sampling, microbiological culture and susceptibility testing

3.3.1 Sampling procedure

From the study area's systematically random selected groundwater sources, a total of 163 samples were taken. Samples were taken from dug wells (39), tube wells with manual pumps (64), and boreholes (60). Samples were carefully taken from groundwater sources using sterile plastic 125-ml bottles so as not to contaminate the samples (Kempthorne, 2006). The procedures for sampling varied based on the kind of facility. The tube was thoroughly flushed using the hand pump, which pumped water for a minute. The bottle was then opened, making sure that just the cap's outside edge was gripped, and filled to the 125 ml mark. The borehole to the nearby tap that was linked to the pump was sampled using an identical method to that which was used to gather samples from tube wells using handpumps. A cup and a 25-meter-long line were used to gather a water sample from a well that had been dug. Without making contact with the well's bottom or sidewalls, the cup dropped into the well gradually. A sample collecting bottle was filled with water and the cup was elevated. Within six hours of collection, all samples were placed in a cool box with ice packs and ready to be transported to the Ifakara Health Institute's Bagamoyo laboratory.

3.3.2 Groundwater sanitary inspection

To determine the levels of contamination risk related with probable sources of microbial contamination and to identify 163 groundwater sources, a survey of sanitary risk assessment was conducted. The inspection method uses a checklist of variables related to sanitation features that have an impact on groundwater and it is based on WHO groundwater sanitary inspection recommendations (WHO, 1997). Physically evaluating the groundwater sources, examining the nearby area, and recording the status as “Yes” or “No” for each risk factor identified were all part of the process. Considered the degree of protection, proximity, and placement in relation to pit latrines that were either uphill or downhill. Whereas a "No" response demonstrated the lack of a contamination risk factor, a "Yes" response indicated its presence. Each groundwater source's sanitary score was calculated by adding up all of the risk factors that are currently in place. In order to determine the groundwater source contamination risk levels, hygienic ratings were finally classified. Four risk categories were established for contamination: extremely high range from 9 to 11, high from 6 to 8, intermediate from 3 to 5, and low from 0 to 2.

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

Sterile MicroLab Scientific Gridded MCE membrane filters with a 47 mm diameter and 0.45 µm pore size were used in Aqua Safe membrane filtration systems to filter 100 ml water samples. These membrane filters were then placed on CHROMagar CCA plates that were purchased from CHROMagar (4 Place du 18 Juin 1940, 75006 Paris, France) and incubated for a full day at 37 °C. In order to support the objectives of this investigation, the culture of groundwater samples onto CHROMagar was done expressly to capture *E. coli* growth. As a result, the presence of *E. coli* isolates was indicated by the detection of metallic blue to violet colonies in the agar. Throughout the procedure, the *E. coli* strain ATCC 25922 was used as a reference and control.

3.3.4 Antibiotic susceptibility test of *E. coli* isolates

Twelve different antibiotics were tested on *E. coli* isolates using the disc diffusion method (CLSI, 2023) to determine their susceptibility. The selection of antibiotics was intended to encompass the majority of antibiotic classes that are currently available for treating *E. coli* infections. Amoxicillin-clavulanic acid (30 µg), Trimethoprim-sulfamethoxazole (30 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Nalidixic acid (30 µg), Nitrofurantoin 300 µg, Cefazolin (30 µg), Ampicillin-sulbactam (20 µg), Ciprofloxacin (5µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), and Cefepime (30 µg) were among the antibiotics. Using a sterile 10µl loop, *E. coli* isolates with a McFarland turbidity of 0.5 were produced in normal saline and then dispersed onto Muller Hinton II agar Petri dishes (Liofilchem (R) S.r.l. via Scozia, 64026 Roseto degli Abruzzi {TE} Italy). A sterile cotton swab was also used to transfer and apply the bacterial suspension onto the surface of the Mueller-Hinton agar plate. The control strain, *E. coli* ATCC 25922, was cultured for eighteen hours at 37°C in two plates containing six discs each. In order to determine possible resistance in *E. coli* isolates, a ruler was used to measure the zone diameter for each antibiotic disc in accordance with CLSI recommendations, and the data were entered into an Excel sheet.

3.4 Multidrug resistance in *E. coli* isolates

Resistance of *E. coli* isolates to at minimum one cephalosporin antibiotic (Cefazolin, Cefuroxime, Ceftriaxone, and Cefepime) in combination with at minimum one of the other antibiotics tested was referred to as multidrug resistance.

3.5 Multiple Antibiotic Resistance Index (MAR)

The multiple antibiotic resistance index was calculated by dividing the number of antibiotics to which *E. coli* isolates showed resistance by the total number of antibiotics tested. (Krumperman, 1983). In regions where antibiotics are highly used, a MAR index value larger than 0.2 indicates a higher risk of groundwater contamination (Davis & Brown, 2016). The MAR index was utilized in this investigation to recommend groundwater source exposure to regions with high antibiotic consumption and the possibility of outbreaks of resistant *E. coli* in groundwater facilities.

3.6 Data management and analysis

Daily, sanitary inspection data was collected using Google Forms and saved in Google Drive. Laboratory findings were entered into Excel once a week. After that, the information was exported to Microsoft Excel (version 2108) and combined with the results of the analysis of antibiotic resistance. The data was restructured and thoroughly cleaned to identify duplication and structural problems in order to assure accuracy. For the thorough analysis of the data, STATA 17 (Stata Corp 4905 Lakeway Drive College Station, Texas 77845 USA) was used. The main goal was to find out how common resistance was in *E. coli* with reference to the 12 antibiotics that were tested. The prevalence of resistance in *E. coli* with respect to the tested antibiotics was illustrated using descriptive analytic approaches, which were also used to assess the sanitary risk level related to groundwater sources and the multiple antibiotic resistance indices of the *E. coli* isolates. Additionally, the spread of groundwater sources polluted with resistant *E. coli* was displayed using Google Earth Pro (Data SIO, NOAA, U.S. Navy, NGA, GEBCO Image Landsat/Copernicus image IBCAO).

3.7 Permission and Ethical consideration

This study found Ethical approval from the Ifakara Health Institute Review Board (IHI/IRB/No: 23-2023). Since the study samples were from groundwater sources, informed consent was obtained from the owner or person supervising the water facility. Owners and supervisors were asked for permission to sample water from their sources and since were voluntary issue they were not forced to participate. Water samples were collected solely from individuals who consented to participate, while those who declined were allowed to do so without any objection, despite being informed about the study's purpose and importance. They were assured that the information they provided would remain confidential and would only be used for scientific research purposes, with no disclosure to any other parties. However, before sampling groundwater sources, a permission letter obtained from District Executive Director to conduct sampling activities to the selected wards within Bagamoyo district council. In addition, local government leaders from wards fully participated (ward executive officer and kitongoji chairpersons) in sample collection activities.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Description of samples, *E. coli* isolates and resistance rates in groundwater

Among the groundwater samples examined for the presence of *E. coli* isolates, 44.8% (73 out of 163) tested positive for *E. coli*. Antibiotic susceptibility testing revealed that 83.6% (61 out of 73) of these isolates displayed resistance to one or more antibiotics, with 70% (51 out of 73) exhibiting multidrug resistance. Notably, there was a significant prevalence of resistant *E. coli* in samples from dug wells, with 89% (24 out of 39) showing resistance. Additionally, Table 1 illustrates the high occurrence of multidrug-resistant *E. coli* in samples from both boreholes and dug wells.

Table 1*:* Descriptive statistics of antibiotic resistance in *E. coli* from groundwater sources

Variable	Total	Sample type Borehole n (%)	 Tube well n (%)	 Dug well n (%)
No. of samples collected	163	60(36.8)	64 (39.3)	39 (23.9)
No. of sample with *E. coli* isolates	73	31 (52)	15 (23)	27 (69)
No. of samples with resistant *E. coli* isolates	61	25 (81)	12 (80)	24 (89)
No. of samples with multi drug-resistant *E. coli* isolates	51	23 (74)	8 (53)	20 (74)

4.2 Profile of antibiotic resistance in *E. coli* isolates

After undergoing antibiotic resistance testing, *E. coli* isolates showed elevated resistance levels, with 56.2% (41 out of 73) resistant to cefazolin, 54.8% (40 out of 73) resistant to nitrofurantoin, 45.2% (33 out of 73) resistant to amoxicillin-clavulanic acid, and 42.5% (31 out of 73) resistant to ceftriaxone. Analysis of antibiotic resistance across various groundwater facility types revealed a notably high resistance rate of 70.4% (19 out of 27) to nitrofurantoin in *E. coli* isolates from dug wells. Furthermore, 58.1% of *E. coli* isolates from boreholes and 46.7% from tube wells with hand pumps exhibited high resistance rates to cefazolin. However, none of the *E. coli* isolates from tube wells with hand pumps or dug wells showed resistance to ciprofloxacin, and all isolates from boreholes were susceptible to gentamicin, as outlined in Table 2.

Table 2: Antibiotic resistance profile in *E. coli* to the tested antibiotics

Antibiotics	Overall N=73 resistance n (%)	Antibiotic resistance from contaminated groundwater facility type		
		Borehole (31) n (%)	Tube well/hand pump (15) n (%)	Dug well (27) n (%)
Cefazolin	41 (56.2)	18 (58.1)	7 (46.7)	16 (59.3)
Ceftriaxone	31 (42.5)	12 (38.7)	4 (26.7)	15 (55.6)
Ciprofloxacin	3 (4.1)	3 (9.7)	None	None
Cefepime	9 (12.3)	3 (9.7)	2 (13.3)	4 (14.8)
Amoxicillin clavulanic acid	33 (45.2)	14 (45.2)	6 (40.0)	13 (48.1)
Nalidixic acid	11 (15.1)	3 (9.7)	1 (6.7)	7 (25.9)
Trimethoprim-sulfamethoxazole	21 (28.8)	7 (22.6)	5 (33.3)	9 (33.3)
Gentamicin	2 (2.7)	None	1 (6.7)	1 (3.7)
Ampicillin -sulbactam	16 (21.9)	7 (22.6)	2 (13.3)	7 (25.9)
Chloramphenicol	27 (36.99)	13 (41.9)	5 (33.3)	9 (33.3)
Cefuroxime	14 (19.2)	6 (19.4)	1 (6.7)	7 (25.9)
Nitrofurantoin	40 (54.8)	16 (51.6)	5 (33.3)	19 (70.4)

4.3 Multiple antibiotic resistance index

The Multiple Antibiotic Resistance (MAR) index is a straightforward tool used to assess whether identified isolates originate from areas with significant or minimal antibiotic usage. A MAR index exceeding 0.2 suggests a high probability of contamination from environments where antibiotics are frequently employed (Davis & Brown, 2016). In the case of *E. coli* isolates from the Bagamoyo groundwater source, the MAR index was recorded at 0.28. In contrast, MAR indices for isolates from dug wells varied between 0 and 0.75, while those from boreholes and tube wells with hand pumps ranged from 0 to 0.58.

4.4 Spatial distribution of groundwater contaminated with resistant *E. coli*

Figure 3 illustrates the spatial distribution of boreholes, tube wells with hand pumps, and dug wells contaminated with resistant *E. coli* in the Bagamoyo District. The figure reveals significant contamination of groundwater sources, predominantly in the central part of the district. Notably, tube wells with hand pumps show a higher rate of contamination by resistant *E. coli* compared to boreholes within this central area.

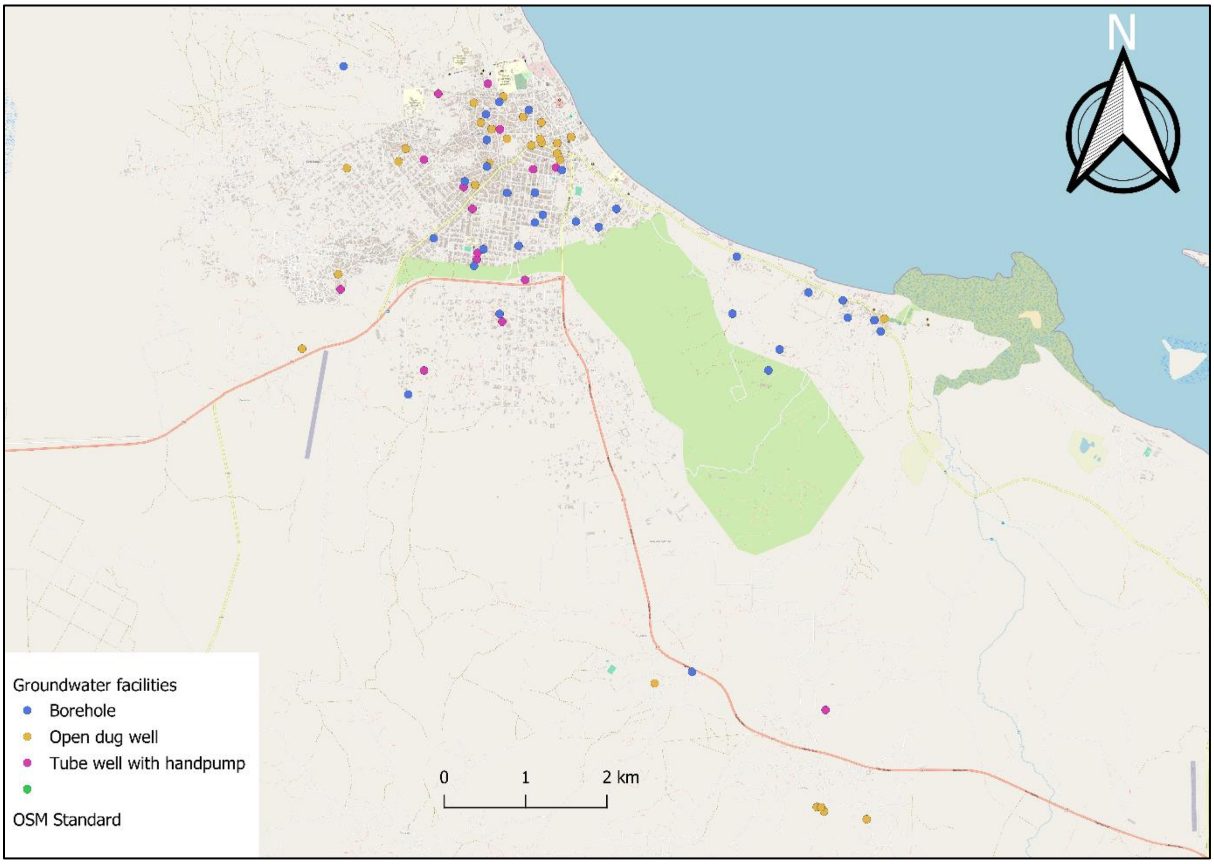

Figure 3: Spatial distribution of boreholes, tube wells with hand pumps, and dug wells contaminated with resistant *E. coli*

4.5 Sanitary risk inspection of the groundwater sources

A total of 163 groundwater sources, comprising 60 boreholes, 39 dug wells, and 64 tube wells with hand pumps, were surveyed to assess sanitary contamination risks in the study area. The majority of the dug wells assessed exhibited poor sealing to the wall lining, allowing water to seep through (35, representing 89.7% of all dug wells), with 15.4% (6/39) located near defective or broken drainage channels leading to ponding. The study revealed that 65.6% (42/64) of tube wells with hand pumps were positioned in areas with inadequate drainage, resulting in stagnant water within 2 meters, while 6.3% (4/64) were situated close to other sources of pollution, such as disposal pits or waste collection areas within 10 meters from the tube wells. Additionally, 45% (27/60) of borehole pump houses were located within 15 to 20 meters from latrines, and no uncapped wells were observed within 15 to 20 meters of boreholes. Various other sanitary features observed around groundwater are detailed in Table 3.

Table 3: Sanitary characteristics of groundwater source facilities (N=163)

Sanitary factor	Dug well (39) n (%)	Tube well (64) n (%)	Sanitary factor	Borehole (60) n (%)
Rope/pump/bucket at risk of contamination	34 (87.2)	35 (54.7)	A latrine within 15–20m	27 (45)
Latrine within 10 m	11 (28.2)	13 (20.3)	-	
Inadequate wall lining seal	35 (89.7)	-	Damaged fencing	3 (5)
Concrete floor < 1 m	22 (56.4)	13 (20.3)	Permeable pump house floor	7 (11. 7)
sources of pollution within 10 m	12 (30.8)	4 (6.3)	Other sources of pollution 10m	5 (8.3)
Inadequate parapet wall	22 (56.4)	9 (14.1)	Unsanitary sealed	15 (25)
Poor drainage within 2 m	22 (56.4)	42 (65.6)	Nearest percolating pit latrine	18 (30)
Cracks in the concrete floor	26 (66.7)	24 (37.5)	uncapped well within 15–20m	-
The nearest latrine uphill	10 (25.6)	12 (18.8)	-	-
faulty/broken drainage channel	6 (15.4)	7 (10.9)	Faulty/broken drainage	7 (11. 7)

Note: n (%) =number of borehole, tube wells/handpump or dug wells that observed to have sanitary factor (Percentage of borehole, tube wells/handpump or dug wells were sanitary factor was observed)

4.6 Groundwater sources contamination risk level

To determine the contamination risk levels of the facilities, 60 boreholes, 64 tube wells with hand pumps, and 39 dug wells were examined. None of the boreholes were categorized as having a "Very high" or "High" contamination risk based on our assessment. Among the boreholes inspected, 44 (73.3%) were classified as having a "low" contamination risk. Regarding the tube wells with hand pumps, only 6.3% (4/64) were found to have a "Very high" risk of contamination, while 21 (32.8%) had varying levels of "high" and "low" contamination risks. Furthermore, the study revealed that 5.1% (2/39) of dug wells were rated as having a "low" contamination risk, whereas 46.2% (18/39) were deemed to have a "high" contamination risk. Table 4 provides a summary of the contamination risk levels across different groundwater source facilities.

Table 4: Statistical description of contamination risk level by groundwater source facilities

Contamination risk level	**Groundwater source facilities type**		
	Boreholes (60) n (%)	**Tube well/hand pump (64)** n (%)	**Dug well (39)** n (%)
Very high	None	4 (6.3)	4 (10.3)
High	None	21 (32.8)	18 (46.2)
Intermediate	16 (26.7)	18 (28.1)	15 (38.5)
Low	44 (73.3)	21 (32.8)	2 (5.1)

Note: n (%) =Number of borehole, tube wells/handpump or dug wells at specific contamination risk level (Percentage of borehole, tube wells/handpump or dug wells at contamination risk level

4.7 Distribution of antibiotic-resistant *E. coli* across diverse contamination risk levels

The research found that 83.6% of all groundwater isolates were resistant to one or more antibiotics. However, the distribution of these resistant isolates in groundwater varies depending on the contamination category. Specifically, the study indicates that all isolates from groundwater sources classified as being at very high risk of contamination were resistant to the tested antibiotics, while 77.8% (21 out of 27) of isolates from groundwater sources categorized as having low contamination risk showed resistance. The distribution of resistant *E. coli* across contamination risk categories is detailed in Table 5.

Table 5: Antibiotic-resistant *E. coli* in groundwater categorized by the level of sanitary risk level

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

4.8 Discussion

The results of this study reveal a concerning prevalence of *Escherichia coli* contamination and antibiotic resistance in groundwater sources within Bagamoyo District. Nearly half (44.8%) of the 163 groundwater samples tested positive for *E. coli,* with a striking 83.6% of these isolates displaying resistance to at least one antibiotic and 70% showing multidrug resistance. Dug wells were particularly vulnerable, with 69% of them contaminated with *E. coli* and 89% of those isolates showing resistance. This trend suggests that the type and condition of groundwater sources significantly influence microbial contamination levels. Notably, multidrug-resistant *E. coli* was detected across all groundwater types, including boreholes and tube wells, further emphasizing widespread environmental exposure to antibiotics or antibiotic-resistant bacteria. The Multiple Antibiotic Resistance (MAR) index, which reached as high as 0.75 in dug wells, supports the likelihood of contamination from areas with high antibiotic use.

Spatial analysis and sanitary inspection findings reinforce the microbiological data, indicating that poor infrastructure and inadequate protection measures are strongly linked to contamination. For example, the central region of the district showed a higher number of groundwater sources contaminated with resistant *E. coli,* especially in tube wells and dug wells lacking proper drainage or located near pollution sources. Sanitary risk assessments revealed that only 5.1% of dug wells had low contamination risk, while a significant number (46.2%) were in the high-risk category. Alarmingly, even groundwater sources classified as having "low" contamination risk were not immune, with 77.8% of *E. coli* isolates from these sources exhibiting antibiotic resistance. This finding highlights the persistence and possible environmental dissemination of resistant bacteria regardless of conventional sanitary risk classification, highlighting the urgent need for improved water source protection and antibiotic stewardship in rural Tanzanian communities.

Investigating antibiotic resistance in groundwater is crucial to understanding the spread of resistant bacteria and how human infections can occur. This issue arises largely due to the misuse of antibiotics in animal husbandry, and human medicine, significantly contributing to the development of resistant bacteria (Manyi-Loh *et al.*, 2018; Salam *et al.*, 2023; Van *et al.*, 2020). Such misuse has resulted in the accumulation of antimicrobial agents in the environment, encouraging the selection of antibiotic-resistant genes (Serwecińska, 2020). Although many global studies have been conducted, research on the prevalence of antibiotic resistance in groundwater sources in Tanzania remains limited. In Bagamoyo, groundwater is crucial for maintaining a steady water supply for the community but is vulnerable to contamination from sanitary facility effluents. This contamination issue is prevalent in many developing nations, where resistant bacteria have been frequently reported (Aslan *et al.*, 2018; Bojar *et al.*, 2021; Praveenkumarreddy *et al.*, 2020). The objective of this study is to examine the occurrence of resistant *E. coli* and identify sanitary risk factors contributing to groundwater contamination in Bagamoyo. Ultimately, the goal is to enhance public health management to safeguard human health.

In this study, most samples were taken from boreholes and tube wells with hand pumps, followed by samples from dug wells. It was found that the highest rate of *E. coli* contamination occurred in boreholes and dug wells. Both types of groundwater sources exhibited high rates of contamination with resistant *E. coli*. However, boreholes and dug wells showed elevated rates of multidrug-resistant *E. coli*. This contrasts with the findings of Odonkor and Addo (2018), who observed a higher rate of multidrug-resistant *E. coli* in dug wells compared to boreholes.

The research revealed that 44.8% of the sampled water contained *E. coli* isolates, with 83.6% of these isolates showing resistance to at least one antibiotic and 70% displaying multidrug resistance, resulting in a MAR index of 0.28. The rise in antibiotic resistance among *E. coli* is becoming increasingly concerning for public health. Various studies worldwide have underscored the widespread occurrence of multidrug-resistant *E. coli* in water sources. For instance, a study conducted in Morocco reported a 68.8% prevalence of multidrug-resistant *E. coli* in groundwater (Tahri *et al.*, 2021). Another study in Cameroon found that 73.7% of *E. coli* isolates from hand-dug wells and boreholes were antibiotic-resistant (Tangwa *et al.*, 2019). Additionally, research in India indicated that 75% of *E. coli* isolates from groundwater sources were multidrug-resistant (Sharma *et al.*, 2017). The prevalence observed in the current study is slightly higher than that reported in these countries. This difference could be attributed to variations in human activities, such as animal husbandry, farming, and poultry keeping, which contribute to the expansion and spreading of resistance in water sources (Ayukekbong *et al.*, 2017).

The study also examined the MAR index of the sampling sources and found it to be above 0.2, indicating that these groundwater sources are in areas with high antibiotic consumption. The MAR index is often used to assess whether isolates come from regions with significant antibiotic use (Al-Badaii & Abdul Halim, 2021; Sandhu *et al.*, 2016; Titilawo *et al.*, 2015). Several factors could contribute to the contamination of groundwater sources with resistant *E. coli* in Bagamoyo. Notably, proximal sanitary factors present a risk of contamination. For instance, many dug wells were observed to have inadequate wall lining seals, boreholes were located near pit latrines and were unsanitary sealed, and tube wells with hand pumps were situated in areas with poor drainage.

The susceptibility tests conducted in this study showed that *E. coli* isolates displayed the highest resistance rates to cefazolin, followed by nitrofurantoin, amoxicillin-clavulanic acid, ceftriaxone, and chloramphenicol. This resistance likely stems from the misuse or overuse of these antibiotics within the community, leading to the contamination of groundwater through the percolation of sewage from pit latrines and other sanitary facilities (Martiny *et al.*, 2011; Muraleedharan *et al.*, 2019; Skandalis *et al.*, 2021). This raises significant concerns about resistant *E. coli* in groundwater sources. Other authors have also reported antibiotic resistance in *E. coli* globally. For example, a study undertaken in South Africa found that *E. coli* isolated from water sources were resistant to amoxicillin-clavulanic acid (96.4%), chloramphenicol (96.4%), and ciprofloxacin (28.6%) (Tula *et al.*, 2018). Additionally, research in Tanzania indicated that Gram-negative bacteria presented high resistance to third-generation cephalosporins (Camara *et al.*, 2023). Another study in Accra, Ghana, reported that *E. coli* isolates from water sources were highly resistant to chloramphenicol and ciprofloxacin, followed by nalidixic acid, gentamicin, nitrofurantoin, and had a MAR index greater than 0.2 (Odonkor & Addo, 2018). In contrast, *E. coli* isolates from Bagamoyo's groundwater showed low resistance to gentamicin and ciprofloxacin, likely due to the infrequent use of these antibiotics in the public, as ciprofloxacin is frequently prescribed for typhoid fever, which is less prevalent in Bagamoyo (Singh *et al.* 2018).

Additionally, the study assessed the rate of groundwater sources at varying risk of contamination level, specifically with resistant *E. coli.* It found a slight difference in *E. coli* contamination rates across all contamination risk levels. Notably, 40.3% of groundwater sources classified as "low contamination risk" were contaminated with *E. coli.* The contamination rates were high for groundwater sources at "intermediate" and "high" contamination risk levels compared to sources at "very high contamination risk levels." This could be due to natural barriers that impede the movement of contaminants. The pattern of contamination observed from this study aligns with the understanding that sanitary risk scores do not always accurately forecast the contamination of groundwater sources (Luby *et al.*, 2008b).

Moreover, researchers in various regions have documented the presence and impact of resistant *E. coli* in sanitation facilities, emphasizing its role in spreading to sources of water. Inappropriate disposal of antibiotics and inadequate sanitation services, especially in informal settlements, exacerbate the spread of resistant *E. coli* (Karimi *et al.*, 2023). Contamination of resistant *E. coli* can contaminate groundwater through cracked aquifers caused by soil openings and (Graham & Polizzotto, 2013). This indicates that a single sanitary factor can significantly contribute to groundwater contamination risk, depending on the proximity and barriers along the contamination path.

In this study, the spatial distribution of groundwater sources contaminated with resistant *E. coli,* primarily concentrated within the Bagamoyo district were evaluated. The visualization of this spatial map underscores the risk posed by antibiotic resistance and the crucial role of residential groundwater sources as reservoirs for resistant *E. coli.* This phenomenon is likely influenced by sanitation and hygiene practices such as feces disposal, solid waste management, bathing, and laundry activities around groundwater sources, as well as inadequate control of runoff within residential areas, which varies across different areas (Essack, 2021; Fletcher, 2015). This finding is consistent with observations made by other researchers, who have highlighted the impact of environmental factors in disseminating resistance within water sources in similar settings to our study (Reddy & Dubey, 2019; F. Singh *et al.*, 2020; Zhang *et al.*, 2020). Such insights can inform targeted interventions and resource allocation aimed at mitigating the spread of resistant pathogens, thereby safeguarding public health in the Bagamoyo District.

Due to limited resources, it was not possible to cluster water sources for instance number of boreholes, tube wells and dug wells and what was then the proportion sampled from each category. While this approach offered a snapshot of the situation, it couldn't track changes over time. Additionally, the study focused mainly on enteric bacteria to gauge resistance in contaminated groundwater, with *E. coli* acting as an indicator for other resistant bacteria. Due to budget constraints, traditional microbiology cultures were used instead of more advanced molecular techniques, which could have provided a deeper understanding of circulating resistant strains. Moreover, the investigation concentrated solely on the prevalence of resistant *E. coli* and didn't explore the potential contributions of birds and playmate animals as reservoirs of resistance.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Detecting *E. coli* in all the water sources indicates faecal contamination due to probably closeness to latrines or runoffs. Although *E. coli* isolates were encountered to all water sources, Boreholes had intermediate to low levels of contamination risk levels.

The study highlights a significant problem of groundwater contamination, with closely half of the tested sources exhibited existence of *E. coli*, and a considerable portion of these isolates demonstrating resistance to multiple antibiotics. Specifically, the research identifies increased resistance in *E. coli* against certain antibiotics like amoxicillin-clavulanic acid, cefazolin, nitrofurantoin, and ceftriaxone. On the other hand, gentamicin and ciprofloxacin display lower resistance levels in *E. coli* isolated. Additionally, the average MAR- index suggests that *E. coli* isolated are prevalent in regions with high antibiotic usage, leading to widespread multidrug resistance among them. Notably, groundwater sources across all contamination risk levels show a high incidence of resistant *E. coli.*

5.2 Recommendations

To address these risks, the study recommends the implementation of a multi-pronged approach. Firstly, there is an urgent need to improve the sanitary construction and regular maintenance of groundwater facilities, especially dug wells and tube wells with hand pumps. This includes sealing wall linings, constructing proper drainage systems, and preventing the proximity of pollution sources such as latrines and waste pits. Secondly, routine microbial and antibiotic resistance monitoring should be incorporated into local water quality surveillance programs, as visual assessments may underestimate contamination risks. Thirdly, public health campaigns should raise awareness about the safe use of antibiotics, promoting rational antibiotic practices to curb the development and spread of resistance. Lastly, policymakers should prioritize investment in sustainable water infrastructure and enforce regulations on sanitation standards to ensure safe water access for all communities.

REFERENCES

AbuAlshaar, A., Piazza, A., Mercato, A., Marchesini, F., Mattioni Marchetti, V., Bitar, I., Hrabak, J., Spalla, M., Pilla, G., Sconfietti, R., & Migliavacca, R. (2022). OXA-244-Producing ST131 *Escherichia coli* From Surface and Groundwaters of Pavia Urban Area (Po Plain, Northern Italy). *Frontiers in Microbiology*, *13*, 1-14.

Adnan, S., Iqbal, J., Maltamo, M., Bacha, M. S., Shahab, A., & Valbuena, R. (2019). A Simple Approach of Groundwater Quality Analysis, Classification, and Mapping in Peshawar, Pakistan. *Environments*, *6*(12), 1-16. https://doi.org/10.3390/environments6120123

Al-Badaii, F., & Abdul Halim, A. (2021). Potential Risk Assessment of Drinking Water Source Exposed to Contamination Using Microbial Indicators and Multiple Antibiotic Resistance Index. *Iranica Journal of Energy & Environment*, *12*(1), 81–92. https://doi.org/10.5829/ijee.2021.12.01.10

Almakki, A., Jumas-Bilak, E., Marchandin, H., & Licznar-Fajardo, P. (2019). Antibiotic resistance in urban runoff. *Science of The Total Environment*, *667*, 64–76. https://doi.org/10.1016/j.scitotenv.2019.02.183

Amos, G. C. A., Hawkey, P. M., Gaze, W. H., & Wellington, E. M. (2014). Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. *Journal of Antimicrobial Chemotherapy*, *69*(7), 1785–1791.

Andrade, L., Boudou, M., Hynds, P., Chique, C., Weatherill, J., & O'Dwyer, J. (2022). Spatiotemporal dynamics of *Escherichia coli* presence and magnitude across a national groundwater monitoring network, Republic of Ireland, 2011–2020. *Science of The Total Environment*, *840*, 1-13. https://doi.org/10.1016/j.scitotenv.2022.156311

Arbab, S., Ullah, H., Weiwei, W., Wei, X., Ahmad, S. U., Wu, L., & Zhang, J. (2021). Comparative study of antimicrobial action of aloe vera and antibiotics against different bacterial isolates from skin infection. *Veterinary Medicine and Science*, *7*(5), 2061–2067.

Ashbolt, N. J., Grabow, W. O., & Snozzi, M. (2001). Indicators of microbial water quality. *Water quality: Guidelines, Standards and Health*, *30*, 289-316.

Aslan, A., Cole, Z., Bhattacharya, A., & Oyibo, O. (2018). Presence of Antibiotic-Resistant *Escherichia coli* in Wastewater Treatment Plant Effluents Utilized as Water Reuse for Irrigation. *Water*, *10*(6), 1-11. https://doi.org/10.3390/w10060805

Ayad, N., Haffaressas, Y., & Mouffok, F. (2021). Groundwater bacteriological quality assessment: Impact of urbanization and agricultural activity. *Water Supply*, *21*(7), 3825–3836. https://doi.org/10.2166/ws.2021.143

Ayukekbong, J. A., Ntemgwa, M., & Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance & Infection Control*, *6*(1), 1-8. https://doi.org/10.1186/s13756-017-0208-x

Azuma, T., Uchiyama, T., Zhang, D., Usui, M., & Hayashi, T. (2022). Distribution and characteristics of carbapenem-resistant and extended-spectrum $β$-lactamase (ESBL) producing *Escherichia coli* in hospital effluents, sewage treatment plants, and river water in an urban area of Japan. *Science of The Total Environment*, *839*, 1-53.

Balasubramanian, N., Sivasubramanian, P., Soundranayagam, J. P., Chandrasekar, N., & Gowtham, B. (2015). Groundwater classification and its suitability in Kadaladi, Ramanathapuram, India using GIS techniques. *Environmental Earth Sciences*, *74*(4), 3263–3285. https://doi.org/10.1007/s12665-015-4394-7

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology*, *13*(5), 310–317. https://doi.org/10.1038/nrmicro3439

Bivins, A., Lowry, S., Murphy, H. M., Borchardt, M., Coyte, R., Labhasetwar, P., & Brown, J. (2020). Waterborne pathogen monitoring in Jaipur, India reveals potential microbial risks of urban groundwater supply. *Npj Clean Water*, *3*(1), 1-10.

Bojar, B., Sheridan, J., Beattie, R., Cahak, C., Liedhegner, E., Munoz-Price, L. S., Hristova, K. R., & Skwor, T. (2021). Antibiotic resistance patterns of *Escherichia coli* isolates from the clinic through the wastewater pathway. *International Journal of Hygiene and Environmental Health*, *238*, 1-8. https://doi.org/10.1016/j.ijheh.2021.113863

Camara, N., Moremi, N., Mghamba, J., Eliakimu, E., Shumba, E., Ondoa, P., & Egyir, B. (2023). Surveillance of antimicrobial resistance in human health in Tanzania: 2016–2021. *African Journal of Laboratory Medicine*, *12*(1), 1-8.

Carrard, N., Foster, T., & Willetts, J. (2019). Groundwater as a Source of Drinking Water in Southeast Asia and the Pacific: A Multi-Country Review of Current Reliance and Resource Concerns. *Water*, *11*(8), 1-20. https://doi.org/10.3390/w11081605

Caudell, M. A., Mair, C., Subbiah, M., Matthews, L., Quinlan, R. J., Quinlan, M. B., Zadoks, R., Keyyu, J., & Call, D. R. (2018). Identification of risk factors associated with carriage of resistant *Escherichia coli* in three culturally diverse ethnic groups in Tanzania: A biological and socioeconomic analysis. *The Lancet Planetary Health*, *2*(11), e489–e497. https://doi.org/10.1016/S2542-5196(18)30225-0

Chique, C., Hynds, P., Burke, L. P., Morris, D., Ryan, M. P., & O'Dwyer, J. (2021). Contamination of domestic groundwater systems by verotoxigenic *escherichia coli* (VTEC), 2003–2019: A global scoping review. *Water Research*, *188*, 1-15 https://doi.org/10.1016/j.watres.2020.116496

Chmielarczyk, A., Wójkowska-Mach, J., Romaniszyn, D., Adamski, P., Helwich, E., Lauterbach, R., Pobiega, M., Borszewska-Kornacka, M., Gulczyńska, E., Kordek, A., & Heczko, P. B. (2014). Mode of delivery and other risk factors for *Escherichia coli* infections in very low birth weight infants. *BMC Pediatrics*, *14*(1), 1-9.

CLSI. (2023). *Performance standards for antimicrobial susceptability testing. clinical and laboratory standards institute.* https://clsi.org/shop/standards/m100/

Coleman, B. L., Salvadori, M. I., McGeer, A. J., Sibley, K. A., Neumann, N. F., Bondy, S. J., Gutmanis, I. A., McEwen, S. A., Lavoie, M., Strong, D., Johnson, I., Jamieson, F. B., & Louie, M. (2012). The role of drinking water in the transmission of antimicrobial-resistant *E. coli. Epidemiology and Infection*, *140*(4), 633–642.

Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S., & Laxminarayan, R. (2018). Anthropological and socioeconomic factors contributing to global antimicrobial resistance: A univariate and multivariable analysis. *The Lancet Planetary Health*, *2*(9), e398–e405. https://doi.org/10.1016/S2542-5196(18)30186-4

Da Silva Peixoto, F., Cavalcante, I. N., & Gomes, D. F. (2020). Influence of Land Use and Sanitation Issues on Water Quality of an Urban Aquifer. *Water Resources Management*, *34*(2), 653–674. https://doi.org/10.1007/s11269-019-02467-6

Davis, R., & Brown, P. D. (2016). Multiple antibiotic resistance index, fitness and virulence potential in respiratory Pseudomonas aeruginosa from Jamaica. *Journal of Medical Microbiology*, *65*(4), 261–271. https://doi.org/10.1099/jmm.0.000229

Dever, L. A., & Dermody, T. S. (1991). Mechanisms of bacterial resistance to antibiotics. *Archives of Internal Medicine*, *151*(5), 886–895.

Ercumen, A., Naser, A. M., Arnold, B. F., Unicomb, L., Colford, J. M., & Luby, S. P. (2017). Can sanitary inspection surveys predict risk of microbiological contamination of groundwater sources? Evidence from shallow tubewells in rural Bangladesh. *American Journal of Tropical Medicine and Hygiene*, *96*(3), 561–568. https://doi.org/10.4269/ajtmh.16-0489

Essack, S. (2021). Water, sanitation and hygiene in national action plans for antimicrobial resistance. *Bulletin of the World Health Organization*, *99*(8), 606–608. https://doi.org/10.2471/BLT.20.284232

Ferrer, N., Folch, A., Masó, G., Sanchez, S., & Sanchez-Vila, X. (2020). What are the main factors influencing the presence of faecal bacteria pollution in groundwater systems in developing countries? *Journal of Contaminant Hydrology*, *228*, 1-28.

Fletcher, S. (2015). Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environmental Health and Preventive Medicine*, *20*(4), 243–252. https://doi.org/10.1007/s12199-015-0468-0

Founou, R. C., Founou, L. L., & Essack, S. Y. (2017). Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PLoS ONE*, *12*(12), 1–18. https://doi.org/10.1371/journal.pone.0189621

Galindo-Méndez, M. (2020). *Antimicrobial resistance in Escherichia coli. In E. Coli Infections-Importance of Early Diagnosis and Efficient Treatment*. IntechOpen. https://www.intechopen.com/chapters/72615

Genter, F., Willetts, J., & Foster, T. (2021). Faecal contamination of groundwater self-supply in low- and middle income countries: Systematic review and meta-analysis. *Water Research*, *201*, 1-10. https://doi.org/10.1016/j.watres.2021.117350

Godman, B., Egwuenu, A., Wesangula, E., Schellack, N., Kalungia, A. C., Tiroyakgosi, C., Kgatlwane, J., Mwita, J. C., Patrick, O., Niba, L. L., Amu, A. A., Oguntade, R. T., Alabi, M. E., Ncube, N. B. Q., Sefah, I. A., Acolatse, J., Incoom, R., Guantai, A. N., Oluka, M., … Brink, A. J. (2022). Tackling antimicrobial resistance across sub-Saharan Africa: Current challenges and implications for the future. *Expert Opinion on Drug Safety*, *21*(8), 1089–1111. https://doi.org/10.1080/14740338.2022.2106368

Graham, J. P., & Polizzotto, M. L. (2013). Pit Latrines and Their Impacts on Groundwater Quality: A Systematic Review. *Environmental Health Perspectives*, *121*(5), 521–530. https://doi.org/10.1289/ehp.1206028

Hacker, J., & Blum-Oehler, G. (2007). In appreciation of Theodor Escherich. *Nature Reviews Microbiology*, *5*(12), 1-1. https://doi.org/10.1038/nrmicro1810

Howard, G., Pedley, S., Barrett, M., Nalubega, M., & Johal, K. (2003). Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda. *Water Research*, *37*(14), 3422-3429. https://doi.org/10.1016/S0043-1354(03)00235-5

Hynds, P. D., Misstear, B. D., & Gill, L. W. (2012). Development of a microbial contamination susceptibility model for private domestic groundwater sources. *Water Resources Research*, *48*(12), 1-13. https://doi.org/10.1029/2012WR012492

Ibekwe, A. M., Murinda, S. E., & Graves, A. K. (2011). Microbiological Evaluation of Water Quality from Urban Watersheds for Domestic Water Supply Improvement. *International Journal of Environmental Research and Public Health*, *8*(12), 4460–4476. https://doi.org/10.3390/ijerph8124460

Indika, S., Wei, Y., Cooray, T., Ritigala, T., Jinadasa, K. B. S. N., Weragoda, S. K., & Weerasooriya, R. (2022). Groundwater-Based Drinking Water Supply in Sri Lanka: Status and Perspectives. *Water*, *14*(9), 1-24. https://doi.org/10.3390/w14091428

Jain, S., Self, W. H., Wunderink, R. G., Fakhran, S., Balk, R., Bramley, A. M., Reed, C., Grijalva, C. G., Anderson, E. J., Courtney, D. M., Chappell, J. D., Qi, C., Hart, E. M., Carroll, F., Trabue, C., Donnelly, H. K., Williams, D. J., Zhu, Y., Arnold, S. R., … Finelli, L. (2015). Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *New England Journal of Medicine*, *373*(5), 415–427.

Johnson, A. P. (2015). Surveillance of antibiotic resistance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670), 1-12.

Johnson, A. P., Speller, D. C., George, R. C., Warner, M., Domingue, G., & Efstratiou, A. (1996). Prevalence of antibiotic resistance and serotypes in pneumococci in England and Wales: Results of observational surveys in 1990 and 1995. *BMJ : British Medical Journal*, *312*(7044), 1454–1456.

Kamara, D., Bah, D., Sesay, M., Maruta, A., Sesay, B. P., Fofanah, B. D., Kamara, I. F., Kanu, J. S., Lakoh, S., Molleh, B., Guth, J., Sagili, K. D., Tavernor, S., & Wilkinson, E. (2022). Evaluation of Drinking Water Quality and Bacterial Antibiotic Sensitivity in Wells and Standpipes at Household Water Points in Freetown, Sierra Leone. *International Journal of Environmental Research and Public Health*, *19*(11), 1-18.

Karimi, K. J., Ngumba, E., Ahmad, A., Duse, A. G., Olago, D., Ndwigah, S. N., Mwanthi, M. A., Ayah, R., & Dulo, S. (2023). Contamination of groundwater with sulfamethoxazole and antibiotic resistant *Escherichia coli* in informal settlements in Kisumu, Kenya. *PLOS Water*, *2*(4), 1-15. https://doi.org/10.1371/journal.pwat.0000076

Katakweba, A. A. S., Muhairwa, A. P., Lupindu, A. M., Damborg, P., Rosenkrantz, J. T., Minga, U. M., Mtambo, M. M. A., & Olsen, J. E. (2018). First report on a randomized investigation of antimicrobial resistance in fecal indicator bacteria from livestock, poultry, and humans in Tanzania. *Microbial Drug Resistance*, *24*(3), 260–268.

Kelly, E., Cronk, R., Fisher, M., & Bartram, J. (2021). Sanitary inspection, microbial water quality analysis, and water safety in handpumps in rural sub-Saharan Africa. *Npj Clean Water*, *4*(1), 1-7. https://doi.org/10.1038/s41545-020-00093-z

Kraupner, N., Ebmeyer, S., Bengtsson-Palme, J., Fick, J., Kristiansson, E., Flach, C. F., & Larsson, D. G. J. (2018). Selective concentration for ciprofloxacin resistance in *Escherichia coli* grown in complex aquatic bacterial biofilms. *Environment International*, *116*(1), 255–268. https://doi.org/10.1016/j.envint.2018.04.029

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*, *46*(1), 165–170. https://doi.org/10.1128/aem.46.1.165-170.1983

Kusi, J., Ojewole, C. O., Ojewole, A. E., & Nwi-Mozu, I. (2022). Antimicrobial Resistance Development Pathways in Surface Waters and Public Health Implications. *Antibiotics*, *11*(6), 1-22. https://doi.org/10.3390/antibiotics11060821

Lanyon, C. W., King, J. R., Stekel, D. J., & Gomes, R. L. (2021). A Model to Investigate the Impact of Farm Practice on Antimicrobial Resistance in UK Dairy Farms. *Bulletin of Mathematical Biology*, *83*(4), 1–31. https://doi.org/10.1007/s11538-021-00865-9

Law, D. (2000). The history and evolution of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. *World Journal of Microbiology and Biotechnology*, *16*(8–9), 701–709. https://doi.org/10.1023/A:1008927820535

Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., Ukoumunne, O. C., & Gaze, W. H. (2018). Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment International*, *114*(July), 326–333. https://doi.org/10.1016/j.envint.2017.11.003

Li, P., Karunanidhi, D., Subramani, T., & Srinivasamoorthy, K. (2021). Sources and Consequences of Groundwater Contamination. *Archives of Environmental Contamination and Toxicology*, *80*(1), 1–10. https://doi.org/10.1007/s00244-020-00805-z

Ligate, F., Ijumulana, J., Ahmad, A., Kimambo, V., Irunde, R., Mtamba, J. O., Mtalo, F., & Bhattacharya, P. (2021). Groundwater resources in the East African Rift Valley: Understanding the geogenic contamination and water quality challenges in Tanzania. *Scientific African*, *13*, 1-14. https://doi.org/10.1016/j.sciaf.2021.e00831

Luby, S. P., Gupta, S. K., Sheikh, M. A., Johnston, R. B., Ram, P. K., & Islam, M. S. (2008a). Tubewell water quality and predictors of contamination in three flood-prone areas in Bangladesh. *Journal of Applied Microbiology*, *105*(4), 1002-1008. https://doi.org/10.1111/j.1365-2672.2008.03826.x

Luby, S. P., Gupta, S. K., Sheikh, M. A., Johnston, R. B., Ram, P. K., & Islam, M. S. (2008b). Tubewell water quality and predictors of contamination in three flood-prone areas in Bangladesh. *Journal of Applied Microbiology*, *105*(4), 1002–1008. https://doi.org/10.1111/j.1365-2672.2008.03826.x

Lyimo, B., Buza, J., Subbiah, M., Smith, W., & Call, D. R. (2016). Comparison of antibiotic resistant *Escherichia coli* obtained from drinking water sources in northern Tanzania: A cross-sectional study. *BMC Microbiology*, *16*(1), 1–10. https://doi.org/10.1186/s12866-016-0870-9

Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules*, *23*(4), 1-48. https://doi.org/10.3390/molecules23040795

Marijani, E. (2022). Prevalence and Antimicrobial Resistance of Bacteria Isolated from Marine and Freshwater Fish in Tanzania. *International Journal of Microbiology*, *2022*, 1–8. https://doi.org/10.1155/2022/4652326

Martiny, A. C., Martiny, J. B. H., Weihe, C., Field, A., & Ellis, J. C. (2011). Functional Metagenomics Reveals Previously Unrecognized Diversity of Antibiotic Resistance Genes in Gulls. *Frontiers in Microbiology*, *2*, 1-11.

Masterton, R. G. (2000). Surveillance studies: How can they help the management of infection? *Journal of Antimicrobial Chemotherapy*, *46*(90002), 53–58.

Mattioli, M. C., Boehm, A. B., Davis, J., Harris, A. R., Mrisho, M., & Pickering, A. J. (2014). Enteric pathogens in stored drinking water and on caregiver's hands in Tanzanian households with and without reported cases of child diarrhea. *PLoS ONE*, *9*(1), 1-11. https://doi.org/10.1371/journal.pone.0084939

Mattioli, M. C., Pickering, A. J., Gilsdorf, R. J., Davis, J., & Boehm, A. B. (2013). Hands and water as vectors of diarrheal pathogens in Bagamoyo, Tanzania. *Environmental Science and Technology*, *47*(1), 355–363. https://doi.org/10.1021/es303878d

Meletis, G., & Bagkeri, M. (2013). Pseudomonas aeruginosa: Multi-drug-resistance development and treatment options. *Infection Control*, *2*, 34–45.

Mgaya, F. X., Matee, M. I., Muhairwa, A. P., & Hoza, A. S. (2021). Occurrence of Multidrug Resistant *Escherichia coli* in Raw Meat and Cloaca Swabs in Poultry Processed in Slaughter Slabs in Dar es Salaam, Tanzania. *Antibiotics*, *10*(4), 1-10. https://doi.org /10.3390/antibiotics10040343

Moremi, N., Mushi, M. F., Fidelis, M., Chalya, P., Mirambo, M., & Mshana, S. E. (2014). Predominance of multi-resistant gram-negative bacteria colonizing chronic lower limb ulcers (CLLUs) at Bugando Medical Center. *BMC Research Notes*, *7*(1), 1–4.

Muraleedharan, C., Talreja, D., Kanwar, M., Kumar, A., & Walia, S. K. (2019). Occurrence of extended-spectrum β-lactamase-producing bacteria in urban Clinton River habitat. *Journal of Global Antimicrobial Resistance*, *16*, 225–235.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals*, *16*(11), 1-54. https://doi.org/10.3390/ph16111615

Neill, J. (2016a). *Tackling drug-resistant infections globally: Final report and recommendations. The review on antimicrobial resistance.* https://www. cabidigitallibrary.org/doi/full/10.5555/20173071720

Neill, J. (2016b). T*ackling drug-resistant infections globally: Final report and recommendations. The review on antimicrobial resistance.* https://www.cabidigitallibrary.org/doi/full/10.5555/20173071720

NESR. (2018). *National Environmental Statistics Report (NESR,2017) Tanzania Mainland*. https://www.nbs.go.tz/nbs/takwimu/Environment/NESR_2017.pdf

Ngasala, T. M., Masten, S. J., & Phanikumar, M. S. (2019). Impact of domestic wells and hydrogeologic setting on water quality in peri-urban Dar es Salaam, Tanzania. *Science of The Total Environment*, *686*, 1238–1250.

Nielsen, K. L., Dynesen, P., Larsen, P., & Frimodt-Møller, N. (2014). Faecal *Escherichia coli* from patients with *E. coli* urinary tract infection and healthy controls who have never had a urinary tract infection. *Journal of Medical Microbiology*, *63*(4), 582–589. https://doi.org/10.1099/jmm.0.068783-0

Nkotagu, H. H. (1989). Geochemistry of shallow groundwater at Kigamboni peninsula along Dar Es Salaam coastal strip Tanzania. *Journal of African Earth Sciences (and the Middle East)*, *9*(3–4), 739–748. https://doi.org/10.1016/0899-5362(89)90060-2

Nnadozie, C. F., & Odume, O. N. (2019). Freshwater environments as reservoirs of antibiotic resistant bacteria and their role in the dissemination of antibiotic resistance genes. *Environmental Pollution*, *254*, 113067. https://doi.org/10.1016/j.envpol.2019.113067

Nowicki, S., deLaurent, Z. R., De Villiers, E. P., Githinji, G., & Charles, K. J. (2021). The utility of *Escherichia coli* as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. *PLoS ONE*, *16*(1), 1-23. https://doi.org/10.1371/journal.pone.0245910

Odjegba, E., Oluwasanya, G., Sadiq, A., & Brion, G. (2020). Sustainability and risk assessment matrix (SRAM): Pathway to water security. *Water Supply*, *20*(7), 2928–2940. https://doi.org/10.2166/ws.2020.196

Odonkor, S. T., & Addo, K. K. (2018). Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. *International Journal of Microbiology, 2018*(1), 1-7.

Odonkor, S. T., & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: An overview. *Microbiology Research*, *4*(1), 5-11. https://doi.org/10.4081/mr.2013.e2

Ombuya, J., Gachoka, K., Dorothy, K., & Mutea, N. (2023). Evaluation of antimicrobial susceptibility of *Escherichia coli* isolated from contaminated areas of Majengo slum in Meru County, Kenya. *F1000Research*, *11*, 1-22.

Omulo, S., Lofgren, E. T., Lockwood, S., Thumbi, S. M., Bigogo, G., Ouma, A., Verani, J. R., Juma, B., Njenga, M. K., Kariuki, S., McElwain, T. F., Palmer, G. H., & Call, D. R. (2021). Carriage of antimicrobial-resistant bacteria in a high-density informal settlement in Kenya is associated with environmental risk-factors. *Antimicrobial Resistance and Infection Control*, *10*(1), 1–12. https://doi.org/10.1186/s13756-021-00886-y

Palmer, G. H., & Buckley, G. J. (2021). *The Health and Economic Burden of Resistance. In Combating Antimicrobial Resistance and Protecting the Miracle of Modern Medicine. National Academies Press (US)*. https://www.ncbi.nlm.nih.gov/books/NBK577288/

Pond, K., King, R., Herschan, J., Malcolm, R., McKeown, R. M., & Schmoll, O. (2020). Improving Risk Assessments by Sanitary Inspection for Small Drinking-Water Supplies—Qualitative Evidence. *Resources*, *9*(6), 1-16. https://doi.org/10.3390/resources9060071

Praveenkumarreddy, Y., Akiba, M., Guruge, K. S., Balakrishna, K., Vandana, K. E., & Kumar, V. (2020). Occurrence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants of South India. *Journal of Water, Sanitation and Hygiene for Development*, *10*(1), 48–55. https://doi.org/10.2166/washdev.2020.051

Prüss-Ustün, A., Bartram, J., Clasen, T., Colford, J. M., Cumming, O., Curtis, V., Bonjour, S., Dangour, A. D., De France, J., Fewtrell, L., Freeman, M. C., Gordon, B., Hunter, P. R., Johnston, R. B., Mathers, C., Mäusezahl, D., Medlicott, K., Neira, M., Stocks, M., … Cairncross, S. (2014). Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: A retrospective analysis of data from 145 countries. *Tropical Medicine and International Health*, *19*(8), 894–905.

Ramatla, T., Ramaili, T., Lekota, K. E., Ndou, R., Mphuti, N., Bezuidenhout, C., & Thekisoe, O. (2023). A systematic review and meta-analysis on prevalence and antimicrobial resistance profile of *Escherichia coli* isolated from water in africa (2000–2021). *Heliyon*, *9*(6), e16123. https://doi.org/10.1016/j.heliyon.2023.e16123

Rao, S. M., Sekhar, M., & Raghuveer Rao, P. (2013). Impact of pit-toilet leachate on groundwater chemistry and role of vadose zone in removal of nitrate and *E. coli* pollutants in Kolar District, Karnataka, India. *Environmental Earth Sciences*, *68*(4), 927–938. https://doi.org/10.1007/s12665-012-1794-9

Reddy, B., & Dubey, S. K. (2019). River Ganges water as reservoir of microbes with antibiotic and metal ion resistance genes: High throughput metagenomic approach. *Environmental Pollution (Barking, Essex: 1987)*, *246*, 443–451.

Reygaert, W. C. (2016). Insights on the antimicrobial resistance mechanisms of bacteria. *Advances in Clinical and Medical Microbiology, 2*(005), 1-11.

Ripanda, A. S., Rwiza, M. J., Nyanza, E. C., Miraji, H., Bih, N. L., Mzula, A., Mwega, E., Njau, K. N., Vuai, S. A. H., & Machunda, R. L. (2023a). Antibiotic-resistant microbial populations in urban receiving waters and wastewaters from Tanzania. *Environmental Chemistry and Ecotoxicology*, *5*, 1–8. https://doi.org/10.1016/j.enceco.2022.10.003

Ripanda, A. S., Rwiza, M. J., Nyanza, E. C., Miraji, H., Bih, N. L., Mzula, A., Mwega, E., Njau, K. N., Vuai, S. A. H., & Machunda, R. L. (2023b). Antibiotic-resistant microbial populations in urban receiving waters and wastewaters from Tanzania. *Environmental Chemistry and Ecotoxicology*, *5*, 1–8. https://doi.org/10.1016/j.enceco.2022.10.003

Roberts, T., Dahal, P., Shrestha, P., Schilling, W., Shrestha, R., Ngu, R., Huong, V. T. L., van Doorn, H. R., Phimolsarnnousith, V., Miliya, T., Crump, J. A., Bell, D., Newton, P. N., Dittrich, S., Hopkins, H., Stepniewska, K., Guerin, P. J., Ashley, E. A., & Turner, P. (2022). Antimicrobial resistance patterns in bacteria causing febrile illness in Africa, South Asia, and Southeast Asia: A systematic review of published etiological studies from 1980-2015. *International Journal of Infectious Diseases*, *122*, 612–621. https://doi.org/10.1016/j.ijid.2022.07.018

Rubino, F., Corona, Y., Pérez, J. G. J., & Smith, C. (2019). Bacterial contamination of drinking water in guadalajara, Mexico. *International Journal of Environmental Research and Public Health*, *16*(1), 1-11. https://doi.org/10.3390/ijerph16010067

Salam, Md. A., Al-Amin, Md. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. A. (2023). *Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. In Healthcare.* MDPI. https://doi.org/10.3390/healthcare11131946

Sanderson, C. E., Fox, J. T., Dougherty, E. R., Cameron, A. D. S., & Alexander, K. A. (2018). The Changing Face of Water: A Dynamic Reflection of Antibiotic Resistance Across Landscapes. *Frontiers in Microbiology*, *9*, 1-13. https://doi.org/10.3389/fmicb.2018.01894

Sandhu, R., Dahiya, S., & Sayal, P. (2016). Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of Acinetobacter species among inpatients. *Indian Journal of Microbiology Research*, *3*(3), 299-304. https://doi.org/10.5958/2394-5478.2016.00064.9

Saria, J. A., & Thomas, I. M. (2012). Water quality in selected shallow wells in Dar es Salaam. *Huria: Journal of the Open University of Tanzania*, *11*, 13-24.

Serwecińska, L. (2020). Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water*, *12*(12), 1-17.

Sharma, B., Parul, Verma, A. K., Jain, U., Yadav, J. K., Singh, R., & Mishra, R. (2017). Occurrence of multidrug resistant *Escherichia coli* in groundwater of Brij region (Uttar Pradesh) and its public health implications. *Veterinary World*, *10*(3), 293–301.

Shlaes, D. M., Sahm, D., Opiela, C., & Spellberg, B. (2013). The FDA reboot of antibiotic development. *Antimicrobial Agents and Chemotherapy*, *57*(10), 4605–4607. https://doi.org/10.1128/AAC.01277-13

Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology*, *7*(11), 1–22. https://doi.org/10.3389/fmicb.2016.01728

Singh, F., Hirpurkar, S. D., Rawat, N., Shakya, S., Kumar, R., Kumar, S., Meena, R. K., Rajput, P. K., & Kumar, J. (2020). Carbapenemase and ESBL genes with class 1 integron among fermenting and nonfermenting bacteria isolated from water sources from India. *Letters in Applied Microbiology*, *71*(1), 70–77. https://doi.org/10.1111/lam.13228

Singh, N. K., Bezdan, D., Checinska Sielaff, A., Wheeler, K., Mason, C. E., & Venkateswaran, K. (2018). Multi-drug resistant Enterobacter bugandensis species isolated from the International Space Station and comparative genomic analyses with human pathogenic strains. *BMC Microbiology*, *18*(1), 1-13. https://doi.org/10.1186/s12866-018-1325-2

Skandalis, N., Maeusli, M., Papafotis, D., Miller, S., Lee, B., Theologidis, I., & Luna, B. (2021). Environmental Spread of Antibiotic Resistance. *Antibiotics*, *10*(6), 1-14.

Sonola, V. S., Katakweba, A., Misinzo, G., & Matee, M. I. (2022). Molecular Epidemiology of Antibiotic Resistance Genes and Virulence Factors in Multidrug-Resistant *Escherichia coli* Isolated from Rodents, Humans, Chicken, and Household Soils in Karatu, Northern Tanzania. *International Journal of Environmental Research and Public Health*, *19*(9), 1–13. https://doi.org/10.3390/ijerph19095388

Sonola, V. S., Katakweba, A. S., Misinzo, G., & Matee, M. I. N. (2021). Occurrence of Multi-Drug-Resistant *Escherichia coli* in Chickens, Humans, Rodents and Household Soil in Karatu, Northern Tanzania. *Antibiotics*, *10*(9), 1-12.

Subbiah, M., Caudell, M. A., Mair, C., Davis, M. A., Matthews, L., Quinlan, R. J., Quinlan, M. B., Lyimo, B., Buza, J., Keyyu, J., & Call, D. R. (2020a). Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nature Communications*, *11*(1), 1-12. https://doi.org/10.1038/s41467-019-13995-5

Subbiah, M., Caudell, M. A., Mair, C., Davis, M. A., Matthews, L., Quinlan, R. J., Quinlan, M. B., Lyimo, B., Buza, J., Keyyu, J., & Call, D. R. (2020b). Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. *Nature Communications*, *11*(1), 1-12. https://doi.org/10.1038/s41467-019-13995-5

Tahri, L., Hafiane, F. Z., & Fekhaoui, M. (2021). Prevalence and antibiotic resistance of the *Escherichia coli* in the groundwater (Tadla-Morocco). *Groundwater for Sustainable Development*, *13*, 100572. https://doi.org/10.1016/j.gsd.2021.100572

Taneja, N., & Sharma, M. (2019). Antimicrobial resistance in the environment: The Indian scenario. *Indian Journal of Medical Research*, *149*(2), 119–128. https://doi.org/10.4103/ijmr.IJMR_331_18

Tangwa, B. V., Keubou, H., Nfor, E. N., & Ngakou, A. (2019). Antimicrobial Resistance Profile of Bacteria Isolated from Boreholes and Hand Dug Wells Water in Ngaoundere Municipality of Adamawa Region in Cameroon. *Advances in Microbiology*, *9*(7), 629-645. https://doi.org/10.4236/aim.2019.97039

Thacker, S. B., & Berkelman, R. L. (1988). Public health surveillance in the United States. *Epidemiologic Reviews*, *10*, 164–190.

Titilawo, Y., Sibanda, T., Obi, L., & Okoh, A. (2015). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of water. *Environmental Science and Pollution Research*, *22*(14), 10969–10980.

Tula, M. Y., Onyeje, G. A., & John, A. (2018). Prevalence of antibiotic resistant and biofilm producing *Escherichia coli* and Salmonella spp from two sources of water in Mubi, Nigeria. *Frontier in Science*, *8*(1), 18-25.

URT. (2017). *The National Action Plan on Antimicrobial Resistance 2017—2022. Ministry of Health Community Development Gender Elderly and Children.* https://www.who.int/publications/m/item/united-republic-of-tanzania-the-national-action-plan-on-antimicrobial-resistance

Van, T. T. H., Yidana, Z., Smooker, P. M., & Coloe, P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: Pluses and minuses. *Journal of Global Antimicrobial Resistance*, *20*, 170–177. https://doi.org/10.1016/j.jgar.2019.07.031

Ventola, C. L. (2012). The Antibiotics. *Comprehensive Biochemistry*, *11*(4), 181–224. https://doi.org/10.1016/B978-1-4831-9711-1.50022-3

Vermeulen, L. C., Hofstra, N., Kroeze, C., & Medema, G. (2015). Advancing waterborne pathogen modelling: Lessons from global nutrient export models. *Current Opinion in Environmental Sustainability*, *14*, 109–120.

Viban, T. B., Herman, O., Layu, T. C., Madi, O. P., Nfor, E., Kingsly, M. T., Germanus, B., Victor, N., & Albert, N. (2021). Risk Factors Contributing to Microbiological Contamination of Boreholes and Hand Dug Wells Water in the Vina Division, Adamawa, Cameroon. *Advances in Microbiology*, *11*(02), 1-19.

Walsh, T. R. (2013). *T*he interconnectivity between humans, animals, and the environment and the consequences for antimicrobial resistance. *Current Opinion in Microbiology*. 16(5), 559–564.

Wang, Y., Mairinger, W., Raj, S. J., Yakubu, H., Siesel, C., Green, J., Durry, S., Joseph, G., Rahman, M., Amin, N., Hassan, Md. Z., Wicken, J., Dourng, D., Larbi, E., Adomako, L. A. B., Senayah, A. K., Doe, B., Buamah, R., Tetteh-Nortey, J. N. N., … Moe, C. L. (2022). Quantitative assessment of exposure to fecal contamination in urban environment across nine cities in low-income and lower-middle-income countries and a city in the United States. *Science of The Total Environment*, *806*, 1-11. https://doi.org/10.1016/j.scitotenv.2021.151273

World Health Organization. (1996). *Guidelines for drinking-water quality: Health and other supporting criteria (2nd Ed., Vol. 2). World Health Organization.* https://apps.who.int /iris/handle/10665/38551

WHO. (1997). *Sanitary inspection packages [Database]. Water Sanitation and Health.* https://www. who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/ water-safety-and-quality/water-safety-planning/sanitary-inspection-packages

World Health Organization. (2011). *Water safety plan manual: Step-by-step risk management for drinking-water suppliers*. World Health Organization. https://apps.who.int/iris/ handle/10665/75141

World Health Organization. (2014). *Antimicrobial resistance: Global report on surveillance*. World Health Organization. https://apps.who.int/iris/handle/10665/112642

WHO. (2021a). *Antimicrobial resistance. Microbiology Australia.* https://doi.org/10.1071/MA19016

World Health Organization. (2022a). *Guidelines for drinking-water quality (4th Ed., incorporating the 1st and 2nd addenda). World Health Organizat*ion. https://www.who.int/publications/i/item/9789240045064

WHO. (2022b). *Progress on household drinking water, sanitation and hygiene 2000–2020: Five years into the SDGs.* World Health Organization. https://www.who.int/publications/i/item/9789240030846

WHO. (2023). *Drinking-water*. https://www.who.int/news-room/fact-sheets/detail/drinking-water

WHO. (2021b). *10 global health issues to track in 2021 [Campain]. World Health Organization Spotlight*. https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021

WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene. (2020). *Five years into the SDGs: Progress on household drinking water, sanitation and hygiene*. World Health Organization (WHO). http://apps.who.int/bookorders

Zhang, S., Huang, J., Zhao, Z., Cao, Y., & Li, B. (2020). Hospital Wastewater as a Reservoir for Antibiotic Resistance Genes: A Meta-Analysis. *Frontiers in Public Health*, *8*, 1-12. https://doi.org/10.3389/fpubh.2020.574968

RESEARCH OUTPUTS

Published paper

Mduda, J. J., Msuya, H. M., Rugaika, A. M., Mkindi, C. G., & Kimaro, E. G. (2025). Assessment of resistant *Escherichia coli* in groundwater sources and sanitary inspection for contamination risk in Bagamoyo, Tanzania. *Journal of Basic and Environmental Sciences, 26*(4), 27–38. https://doi.org/10.21203/rs.3.rs-4174422/v1

Poster presentation