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# Investigating Antibiotic Susceptibility of Pathogenic Microorganisms in Groundwater from Boreholes and Shallow Wells in T/A Makhwira, Chikwawa

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**Abstract:** Many rural communities in Malawi use groundwater from boreholes and shallow wells for drinking and cooking with limited or no treatment because it is considered as a safe source of water. Contamination of groundwater sources by antimicrobial resistant bacteria renders the water unsafe to use. This study investigated the antibiotic susceptibility of pathogenic microorganisms isolated from groundwater sources in T/A Makhwira, Chikwawa. Water samples were collected from 13 boreholes and 7 protected shallow wells from T/A Makhwira, Chikwawa. *E. coli, Salmonella enterica ssp Arizona, K. pneumoniae, ESBL E. coli* and *ESBL K. pneumoniae* were detected in some water samples. Antibiotic susceptibility tests showed that the isolates had high resistance to Ampicillin (42%) followed by Trimethoprim-sulfamethoxazole (26%), Ciprofloxacin (21%), Doxycycline and Amoxicillin/clavulanic acid (16%). The isolates had a very high sensitivity to Gentamicin (89%). The study revealed that the water from some boreholes and shallow wells in T/A Makhwira is highly contaminated and needs to be treated before consumption. Drinking untreated water from these sources could transfer antibiotic resistant bacteria to humans because the groundwater may act as a vehicle for the transmission of these antibiotic resistant bacteria.

**Keywords:** antibiotic resistant bacteria; groundwater; antimicrobial contamination

## 1. Introduction

Groundwater is considered as a very reliable source of drinking water. About 74.9% of rural communities in Malawi depend on water from boreholes, shallow wells and open wells in their day to day lives [1]. People use groundwater for drinking and cooking with limited or no treatment because it is considered as a safe source of water [2]. However, groundwater can easily get contaminated by various pollutants thereby degrading the quality of the water and rendering it unsafe [2]. Various anthropogenic activities, climate change and other natural processes are potential sources of contaminants and they can significantly threaten the quality of groundwater [3]. Contaminants such as pathogenic microorganisms and antimicrobial resistant bacteria can pollute water through seepage from sewer leaks, poorly constructed septic tanks, pit latrines or improper sewage disposal [4]. Pathogenic bacteria found in water are responsible for various waterborne diseases. For example, gastrointestinal diseases are caused by *Shigella, Salmonella, Campylobacter and Clostridium* [5]. Water borne diseases and pathogenic contamination of water is a great water quality concern. Water borne infections kill about 3.4 million people, mostly children, each year worldwide [5]. In recent years, there has been an increase in the occurrence of antibiotic resistant bacteria in the environment and groundwater sources due to the high usage of antibiotics. The presence of antibiotics in groundwater overtime eventually results in the development of antibiotic resistant bacteria [6,7].

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Antimicrobial resistance is one of the major health problems being faced by the global population [8]. By 2050, it is anticipated that the global cost of antimicrobial-resistant pathogen-related diseases will rise from 700,000 to 10 million fatalities annually and reach USD 100 trillion [9]. Antimicrobial resistance is one of the major health problems being faced by the global population [8]. By 2050, it is anticipated that the global cost of antimicrobial-resistant pathogen-related diseases will rise from 700,000 to 10 million fatalities annually and reach USD 100 trillion [9]. The various complex interactions of antibiotics in the environment have led to the development and spread of antibiotic resistant bacteria [10]. When an infection resulting from antibiotic resistant bacteria occurs, it can be difficult to treat such infection thereby leading to extended periods of illness or even death. For example, *N. gonorrhoea* which used to be treatable by antibiotics such as Penicillins, Tetracyclines, Sulfonamides and Fluoroquinolones has now become resistant to these antibiotics [11]. Contamination of groundwater by pollutants such as organics, pesticides, pharmaceuticals, micro plastics and other emerging contaminants like antibiotics and antibiotic resistant bacteria (ARB), poses a health threat to consumers and even the entire human population [3].

According to United Nations general assembly in 2010, access to clean and safe water for human consumption was declared as a human right. Sustainable development goal number 6, target 6.1 states that "By 2030, achieve universal and equitable access to safe and affordable drinking water for all". "Safe" drinking water means that the water is free of contaminants" [12]. Contamination of groundwater by emerging contaminants like antibiotic resistant bacteria renders the water unsafe for human consumption and has various health effects. Several studies in Malawi have investigated organic contaminants in groundwater and not on emerging contaminants like antimicrobial resistant bacteria [13–18]. A Study evaluating seasonal variation of water quality from shallow wells in Democratic Republic of Congo recommended that "further investigations should be done on emerging contaminants like antibiotics, antibiotic resistant bacteria and antibiotic resistant genes in shallow wells" [19]. Currently, in Malawi there is a knowledge gap on antibiotic resistant bacteria and genes in groundwater. It is for this reason that the study will investigate the antibiotic susceptibility of pathogenic microorganisms isolated in groundwater from boreholes and wells in T/A Makhwira, Chikwawa.

## 2. Materials and Methods

This was a descriptive cross-sectional study where quantitative data was collected and analyzed. Water samples were collected in T/A Makhwira, Chikwawa. 13 water samples were collected from boreholes and 7 from protected shallow wells.

### *2.1. Sample Collection*

The researcher collected water samples from boreholes and shallow wells in sterilized 500ml sampling bottles. The sample boreholes were clearly labelled depending on the source of the sample, BH1, BH2 to BH13 for boreholes and SW1, SW2 to SW7 for shallow wells. The collected samples were placed in a cooler box filled with ice after collection and transported to Kamuzu University of Health Sciences laboratory for analysis. The samples were stored in refrigerators at 4$^{o}$C until all the analysis was completed.

### Enumeration of Bacteria Counts

Total plate count was done using spread plate method. The process involved serial dilutions of water samples in distilled water and spread plating 20µl of the distilled samples on nutrient agar in petri dishes. The cultured plates were incubated at 37°C for 24 hours. Total counts were presented as colony-forming units (cfu) per milliliter (cfu/ml).

*2.2. Isolation of Pathogenic Microorganisms*

Pathogenic microorganisms such as *E. coli, K. pneumoniae, salmonella, Shigella, ESBL E. coli and ESBL K. pneumoniae* were detected using standard methods. Samples enriched with BPW were cultured on selective media such as CHROMagar™ Orientation, ESBL Chromogenic agar and Xylose Lysine Deoxycholate agar (XLD agar) to isolate the pathogenic bacteria in water. Suspected *E. coli* and *Salmonella spp*. colonies were further subjected to biochemical tests using API 20E Kits for confirmation and High resolution melt curve (HRM) PCR was used to confirm suspected *K. pneumoniae* colonies.

*2.3. Antibiotic Susceptibility Testing*

Kirby Bauer disk diffusion method was used to determine the resistance and sensitivity of pathogenic bacteria to various selected antibiotics. The antibiotics disks that were used included: Ampicillin 10µg, Amoxicillin-clavulanic acid 30µg, Gentamicin 10µg, Doxycycline 30µg, Ciprofloxacin 5µg and Trimethoprim- sulfamethoxazole 25µg. Pathogenic bacteria isolated from the water samples were inoculated on Mueller-Hilton agar in the presence of antibiotic disks. The Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility Testing- 30$^{th}$ edition were used for the interpretation of the zone diameter breakpoints.

*2.4. Statistical Analysis*

Data was entered and analyzed using IBM SPSS Statistics version 20. Descriptive statistics were used to generate means and frequencies to describe the data. Independent sample T-tests and fishers exact tests were used to assess the significance of associations at 95% confidence interval. Any *p-value* less than <0.05 was significant.

## 3. Results

A total of 20 groundwater samples were analyzed in this study. The results of the heterotrophic plate count showed that there was a high bacteria count in all the water samples. The total counts ranged from $1.6 \times 10^3$ cfu/ml to $2.35 \times 10^5$ cfu/ml for boreholes and $1.2 \times 10^3$ cfu/ml to $2.65 \times 10^5$ cfu/ml for shallow wells. All the counts were higher than the national [20] and international [8] water quality standards. The mean CFU/ml for boreholes was 90708 cfu/ml and 162314 cfu/ml for shallow wells. Further analysis using independent samples T-test found that there was an insignificant difference between the total counts means for the boreholes and shallow wells ($t = -1.426$, $p = 0.188$).

There was a variation in the presence of the selected pathogenic microorganisms among the water samples. *E. coli* 11(55%) was the most prevalent pathogenic bacteria. *K. pneumoniae* 4(20%) was the second most prevalent pathogenic bacteria followed by *Salmonella* 1(5%). There was a low prevalence of ESBL producing enterobacteriaceae in the water sample. *ESBL E. coli* was present in only 1(5%) of the water samples, i.e., SW3. *ESBL K. pneumoniae* was also present in just 2 (10%) of the water samples i.e., SW3 and SW7. *Shigella* was not detected in any of the water samples.

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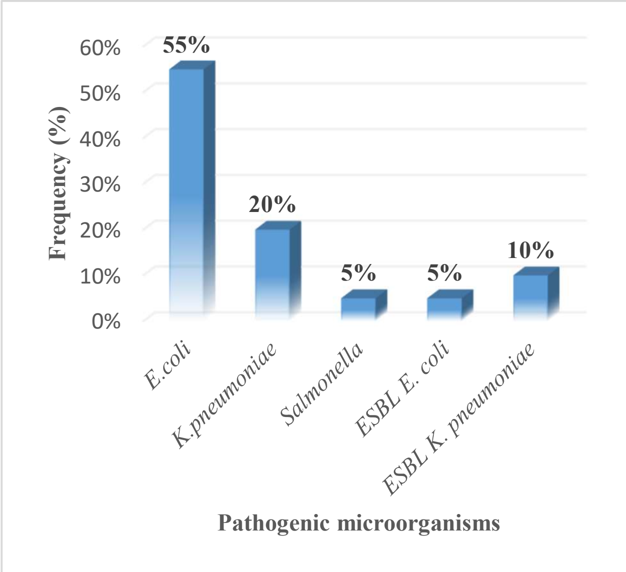


**Figure 1.** Frequency of occurrence of pathogenic microorganisms in groundwater.

Antimicrobial susceptibility testing was done on all the 19 isolates of pathogenic bacteria isolated from the water samples. These isolates included *E. coli*, *Salmonella*, *K. pneumoniae*, *ESBL E. coli* and *ESBL K. pneumoniae*. The isolates had varying sensitivities to different antibiotics. Some isolates were resistant to the antibiotics, others had an intermediate resistance whereas others were sensitive to the antibiotics that were used.

**Table 1.** Antimicrobial susceptibility of bacteria isolates to selected antibiotics.

| | | Antimicrobial susceptibility to selected antibiotics | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample ID** | **Bacteria Isolate** | **AMP** | **SXT** | **AMC** | **DXT** | **CIP** | **CN** |
| BH1 | *E. coli* | S | S | S | S | S | S |
| BH2 | *E. coli* | I | S | I | I | S | S |
| BH7 | *E. coli* | I | S | S | S | S | S |
| BH8 | *E. coli* | I | S | S | S | S | S |
| BH9 | *E. coli* | S | S | S | S | S | S |
| SW1 | *E. coli* | I | S | S | S | S | S |
| SW2 | *E. coli* | I | S | I | S | S | S |
| SW3 | *E. coli* | S | S | S | I | S | S |
| SW4 | *E. coli* | S | S | S | S | R | S |
| SW6 | *E. coli* | S | S | S | S | S | S |
| SW7 | *E. coli* | R | R | S | I | S | S |
| SW3 | *ESBL E. coli* | R | R | R | R | R | R |
| BH11 | *K. pneumoniae* | R | S | S | I | S | S |
| BH2 | *K. pneumoniae* | R | S | S | I | S | S |
| SW1 | *K. pneumoniae* | R | S | R | I | S | S |
| SW6 | *K. pneumoniae* | R | R | S | R | I | S |
| SW3 | *ESBL K. pneumoniae* | R | R | R | I | R | S |
| SW7 | *ESBL K. pneumoniae* | R | R | I | R | R | R |
| SW7 | *Salmonella* | I | S | I | I | I | S |

**KEY**: S- Sensitivity, I- Intermediate Resistance, R- Resistant (S, I and S determined the diameter of the zone of inhibition) AMP- Ampicillin, AMC- Amoxicillin-clavulanic acid, CN-Gentamicin, DXT-Doxycycline, CIP-Ciprofloxacin and SXT- Trimethoprim- sulfamethoxazole.

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High incidence of resistance was observed in Ampicillin (42%), followed by Trimethoprim-sulfamethoxazole (26%) and Ciprofloxacin (21%). There was a 16% resistance in both Amoxicillin-clavulanic acid and Doxycycline. The lowest resistance was observed in Gentamicin (11%).

The isolates exhibited the highest sensitivity in Gentamicin (89%). There was a 78% sensitivity of the isolates to Trimethoprim- sulfamethoxazole and 68% sensitivity to Ciprofloxacin. Amoxicillin-clavulanic acid had a sensitivity of 63%. Lower sensitivity was observed in Ampicillin (26%) and Doxycycline (42%).

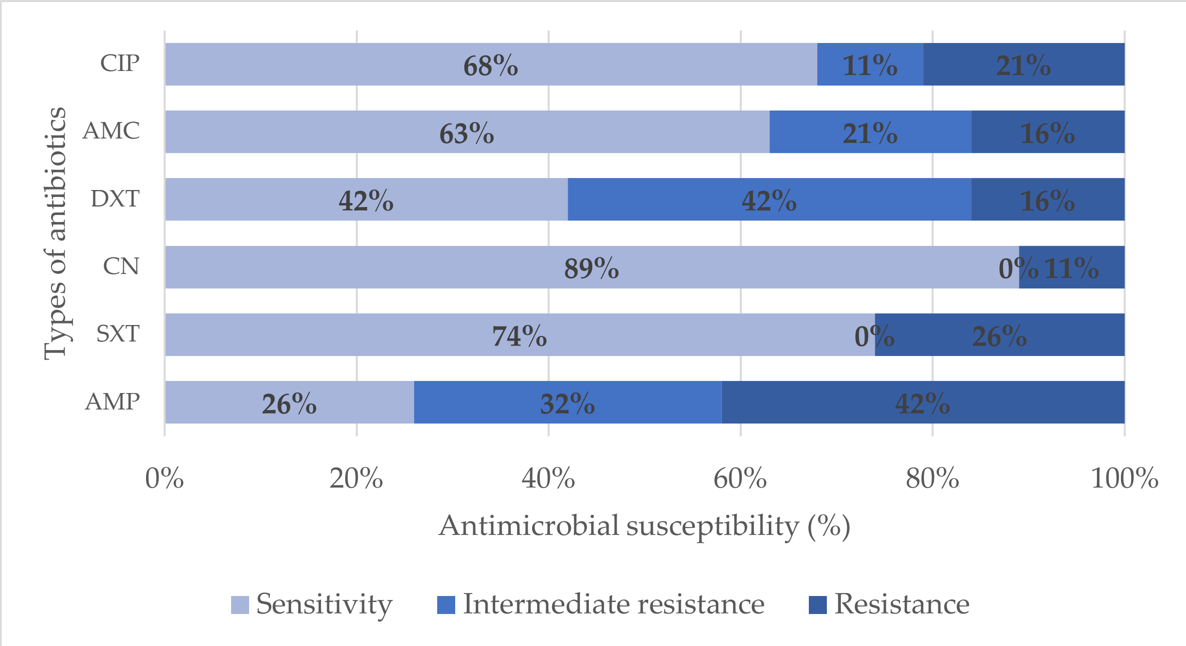


**Figure 2.** Antibiotic susceptibility of bacteria isolates from water samples.

A few isolates (32%) were resistant to more than one antibiotic. Some were resistant to 1 or 2 antibiotics whereas some were resistant to 3 or more antibiotics. ESBL producing enterobacteriaceae were the ones that showed resistance to more than three antibiotics.

**Table 2.** Multi-Drug resistant patterns of isolates from water samples.

| Resistance pattern | Types of antibiotics | Name of isolate |
|---|---|---|
| 1 | AMP, SXT, DXT | SW6_*K. pneumoniae* |
| 2 | AMP, SXT, AMC, CIP | SW3_*ESBL K. pneumoniae* |
| 3 | AMP, SXT, DXT, CIP, CN | SW7_*ESBL K. Pneumoniae* |
| 4 | AMP, SXT, AMC, DXT, CIP, CN | SW3_*ESBL E. coli* |

Fishers exact test showed that there was no significant association between the type of water source and antibiotic susceptibility results as shown in table 3.

**Table 3.** Association between antimicrobial susceptibility and type of water source.

| | Water Source | | |
|---|---|---|---|
| **Antibiotic** | **Borehole**[1], N = 7 | **Shallow well**[1], N = 12 | **p-value**[2] |
| **Ampicillin** | | | 0.8 |
| Intermediate Resistance | 3 (43%) | 3 (25%) | |
| Resistant | 2 (29%) | 6 (50%) | |
| Sensitive | 2 (29%) | 3 (25%) | |
| **Sulfamethoxazole-Trimethoprim** | | | 0.11 |
| Resistant | 0 (0%) | 5 (42%) | |

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| | | | |
|---|---|---|---|
| Sensitive | 7 (100%) | 7 (58%) | |
| **Amoxicillin Clavulanic acid** | | | 0.3 |
| Intermediate Resistance | 1 (14%) | 3 (25%) | |
| Resistant | 0 (0%) | 3 (25%) | |
| Sensitive | 6 (86%) | 6 (50%) | |
| **Doxycycline** | | | 0.4 |
| Intermediate Resistance | 3 (43%) | 5 (42%) | |
| Resistant | 0 (0%) | 3 (25%) | |
| Sensitive | 4 (57%) | 4 (33%) | |
| **Ciprofloxacin** | | | 0.11 |
| Intermediate Resistance | 0 (0%) | 2 (17%) | |
| Resistant | 0 (0%) | 4 (33%) | |
| Sensitive | 7 (100%) | 6 (50%) | |
| **Gentamicin** | | | 0.5 |
| Resistant | 0 (0%) | 2 (17%) | |
| Sensitive | 7 (100%) | 10 (83%) | |

[1]n (%)
[2]Fisher's exact test

## 4. Discussion

All samples collected from boreholes and shallow wells had a CFU/ml that was higher than the recommended national [20] and international [21] water quality standards. This is consistent with results from [22,23] which also found a high colony count above the required standards. This high colony count could be attributed to the proximity of the water points to sanitation facilities and the frequent occurrence of floods in the area. During data collection, it was observed that some of the water points were less than 10 meters away from pit latrines and animal kraals hence the reason for the high colony count. Pit latrines, animal kraals and sporadic presence of wild animals are some of the main contributors to groundwater contamination in the area. T/A Makhwira is an area which has been hit by both cyclone Idai and Freddy in recent years. Flood submergence of both boreholes and shallow wells may also contribute to high microbial contamination. The water samples were collected during rainy season and after part of the area had been affected by cyclone Freddy hence this might have contributed to the high microbial load. Pathogenic bacteria should not be detectable in drinking water [20,21]. The presence of these pathogenic bacteria renders the water unsafe for human consumption and increases the risk of waterborne diseases. *E. coli* was the most prevalent pathogenic bacteria in the water samples that were collected. This result is consistent with findings reported in Malawi [23–25], and Lesotho [26] that attributed high *E. coli* prevalence to proximity of groundwater sources to pit latrines. Studies by have further reported that the presence of *E. coli* in water samples is usually an indication of fecal contamination [27,28]. A study recent study found the highest level of *E. coli* and total coliforms in a water sample from a tube well that was 1m away from a latrine [29]. This is further evidence that proximity to pit latrines is associated with the presence of *E. coli* in groundwater samples. *Salmonella enterica ssp arizonae* was found in one sample out of the 20 water samples that were collected. The presence of this species of *salmonella* in the water is quite alarming as it is not a common human pathogen and it is usually found in the gut flora of snakes [30–32]. This could indicate that there is a potential reservoir of snakes near the protected shallow well where this pathogen was isolated from. This pathogen can be responsible for the occurrence of gastrointestinal diseases such as gastroenteritis in people who consume water from this source. A recent scholarly article discovered that *Salmonella ssp* contamination is higher in dry season than in rainy season and this may explain why salmonella was not found in the other water sources [33]. However, the presence of the *Salmonella enterica ssp arizonae* in the sample from this water source could indicate localized contamination possibly linked to snakes accessing the shallow well. The detection of *K. pneumoniae* in water samples is of significant concern because *K. pneumoniae* is a pathogen that is

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responsible for causing blood, lung and urinary tract infections [34]. Consumption of water contaminated with *K. pneumoniae* puts individuals at risk of these infections. In this study, *K. pneumoniae* was detected in 20% of the water samples. This is consistent with results from [35,36] which also detected *K. pneumoniae* from groundwater samples. In addition to fecal contamination, *K. pneumoniae* can enter groundwater through the use of contaminated irrigation water and the application of manure or wastewater for agricultural reasons. Infiltration and runoff from agricultural areas helps in the spread of these resistant strains [37]. This study also detected *ESBL E. coli* and *ESBL K. pneumoniae* in 3 samples collected from shallow wells. This is in line with results from [38] which detected *ESBL E. coli* in shallow wells and attributed the presence of ESBL producing *E. coli* to fecal contamination. Drinking water from sources contaminated with ESBL producers can expose individuals to these enterobacteriaceae that are well known for being antimicrobial resistant bacteria. Diseases caused by these antimicrobial resistant bacteria are more severe and harder to treat.

This study showed that there was a high incidence of resistance of isolates to Ampicillin (42%). This is consistent with results from [39–41] who also found that isolates had a high resistance to Ampicillin. The high resistance to ampicillin could be because this antibiotic is usually used as a broad-spectrum drug hence the reason why many bacteria species could be resistant to the antibiotic. In the current study, *K. pneumoniae* isolates were found to be resistant to a wide range of antibiotics such as Ampicillin, Trimethoprim-sulfamethoxazole, Amoxicillin clavulanic acid and Doxycycline. This is similar with results from [42] who found that *K. pneumoniae* had 100% resistance to both Ampicillin and Amoxicillin among other antibiotics. In clinical settings, *K. pneumoniae* is a significant pathogen that causes lower respiratory infections and urinary tract infections hence the concern over antibiotic-resistant *K. pneumoniae* is growing [43]. High resistance of *K. pneumoniae* is increasing the burden of antibiotic resistance in humans on a global scale as some of the human infections caused by *K. pneumoniae* are becoming resistant to a number of antibiotics [39]. Some of the *K. pneumoniae* isolates in the current study were found to be multi-drug resistant. This is not in line with results by [44] which found that *K. pneumoniae* isolated from groundwater were not multi-drug resistant. This could be because of the different types of antibiotics that were used during the antimicrobial susceptibility testing in the studies. The current study found ESBL producing enterobacteriaceae isolates in 3 water samples, and all these isolates were multi- drug resistant. Several studies have found that ESBL producing enterobacteriaceae are usually multi-drug resistant. For instance, some scholars found that ESBL producing *E.coli* from shallow wells were resistant to more than 8 antibiotics that were used in antimicrobial susceptibility testing [38]. These 8 antibiotics belonged to classes such as beta lactams, aminoglycosides, fluoroquinolones and sulfonamides. Another study found that 77% of ESBL producing *E. coli* were multi drug resistant. ESBL producing enterobacteriaceae are usually resistant to the antibiotics that are effective in the treatment of non ESBL producing enterobacteriaceae [45]. This could be the reason why ESBL producing enterobacteriaceae are widely considered as antimicrobial resistant bacteria due to their multi-drug resistance patterns.

This study also found that ESBL producing *E. coli* and *K. pneumoniae* isolates were 100% resistant to Ampicillin. Two of the ESBL isolates were also resistant to Amoxicillin- clavulanic acid with one of the isolates having developed intermediate resistance despite clavulanic acid being a Beta-lactames inhibitor. This resistance was expected because Beta-lactam antibiotics cause resistance in Enterobacteriaceae due to production of extended-spectrum beta-lactamases, leading to β-lactam antibiotic resistance worldwide [46]. ESBL-producing strains frequently display co-resistance to additional antibiotic classes, such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, in addition to beta-lactam resistance. Treatment choices are further constrained by this multidrug resistant phenotype [47].

## 5. Conclusions

The study revealed that the water samples from these boreholes and shallow wells contained pathogenic microorganisms that are of public health importance. This revelation shows that most of the water from these groundwater sources is unsafe for consumption hence needs to be treated before

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use. Overall, the study has shown that antimicrobial resistant bacteria are becoming more prevalent in groundwater from boreholes and shallow wells and these groundwater sources have become reserves for these antibiotic resistant bacteria (ARB). Drinking water from these sources could transfer the ARB's to humans as the groundwater acts as a vehicle for the transmission of these Antibiotic resistant bacteria. Therefore, there is a need for regular monitoring of these pathogenic bacteria and antimicrobial resistant bacteria in groundwater to reduce their spread. As a nation, we need to work to protect the integrity of our groundwater and reduce the risks associated with antimicrobial contamination by giving priority to research, putting in place useful policies, and encouraging community involvement in working towards groundwater preservation.

**Author Contributions:** Conceptualization, B.V.B.; methodology, B.V.B and H.W.T.M.; validation, B.V.B, H.W.T.M. and B.T.; formal analysis, B.V.B.; investigation, B.V.B.; resources, B.V.B, H.W.T.M and B.T.; data curation, B.V.B.; writing—original draft preparation, B.V.B.; writing—review and editing, B.V.B, H.W.T.M and B.T.; visualization, B.V.B.; supervision, H.W.T.M and B.T.; All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data is available in a dissertation and can be provided on needs basis.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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