Prevalence of Colistin-Resistant Bacteria in Water Sources in Wa, Upper West Region of Ghana

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ABSTRACT

Background and Objective: Antimicrobial resistance is a major global public health threat, with the highest mortality rate in Sub-Saharan Africa. Colistin is considered the last line of defense against multidrug-resistant Gram-negative pathogens. This study sought to assess the prevalence of colistin-resistant bacteria in water sources in the Wa Municipality of the Upper West Region of Ghana. **Materials and Methods:** A cross-sectional study was conducted, collecting 40 water samples from boreholes, wells and taps in four communities within the Wa Municipality. Samples were cultured and isolates were identified using standard biochemical tests. Colistin susceptibility was assessed using Kirby-Bauer disk diffusion and microbroth dilution techniques, with results interpreted using EUCAST standards. Data was analysed using SPSS version 28. **Results:** Diverse Gram-negative enteric organisms were isolated, including *Citrobacter* species (28.5%), *Klebsiella* species (25%), *Serratia marcescens* (21.4%), and others. Microbroth dilution testing revealed 44.8% of isolates were colistin-resistant, compared to only 4.7% by disk diffusion. Resistant organisms were found across all communities and water sources. Urban areas showed a significant association with colistin resistance (p = 0.049). **Conclusion:** High colistin resistance in Wa water sources indicates sanitation challenges. Improved water treatment and antibiotic stewardship are crucial. Ongoing surveillance is recommended.

INTRODUCTION

The introduction of antibiotics into medicine is arguably the greatest medical achievement of the 20th century[1]. Studies suggest that in addition to making it feasible to treat infections, antibiotics also aid in performing a number of highly advanced medical procedures such as open-heart surgery, cancer treatment and organ transplantation[2,3]. Despite this great achievement, the misuse of antibiotics, among other factors, has led to the challenge of antimicrobial resistance, which is a burden[4-6].

Microbes evolve to withstand antimicrobial treatments, rendering these drugs ineffective. This phenomenon, known as Antimicrobial Resistance (AMR), complicates infection management, potentially escalating disease transmission, morbidity and mortality rates[7,8]. Antimicrobial resistance is a major global public health threat, with the highest mortality rate in sub-Saharan Africa at 27.3 deaths per 100,000 people[9,10]. Severe infections caused by bacteria that are resistant to multiple drugs, especially carbapenem-resistant bacteria, are a threat to last-line antibiotics such as colistin. In the last few years, there has been a gradual increase in the prevalence of colistin resistance[11]. This highlights the need for more studies to understand the challenges of colistin resistance, especially in areas with high AMR-related mortality.

Colistin is an antibiotic used in both human and veterinary medicine. Colistin, classified as a polymyxin antibiotic, is one of only two polymyxins (along with polymyxin B) currently employed in human clinical practice[12-14]. It comprises two bactericidal compounds, polymyxin E1 and E2, which are pentacationic lipopeptides. These molecules target Gram-negative bacteria, compromising their outer membrane integrity and leading to cell death[15]. Healthcare providers primarily prescribe colistin to combat infections caused by bacteria exhibiting multidrug, extensive or pan-drug resistance patterns. It is not fully understood how colistin works, but it is thought to bind to lipopolysaccharides and phospholipids in the outer membrane of Gram-negative bacteria causing membrane disruption and consequently cell death[16].

Studies show that pathogenic and potentially pathogenic bacteria, many of which may have antibiotic resistant genes, are constantly released into water environments with wastewater[17,18]. These genes get inserted into genetic mobile platforms such as plasmids and spread from water to soil[17]. Consequently, water is not only a means of spreading antibiotic-resistant organisms across human and animal populations, but it also introduces resistant genes into natural bacterial ecosystems. Non-pathogenic bacteria in such systems can serve as a repository of resistance genes and mobile platforms[19]. As a habitat for microbes, water can be the source of antibiotic resistance genes, a reservoir for genes that have already been acquired by human pathogens or a place where antibiotic resistance genes can be exchanged between different types of bacteria, indicating the important role water plays in the spread of antibiotic resistance[17,19,20]. However, despite the research in this area, it is still unclear under which circumstances water bacteria are important sources of novel mechanisms of antibiotic resistance.

As a crucial defence against multidrug-resistant Gram-negative pathogens, colistin plays a vital role in treating bacterial infections resistant to various antibiotics, including mcr-1 cephalosporins, quinolones, aminoglycosides and carbapenems[21]. However, the discovery of plasmid-mediated colistin resistance genes in carbapenem-resistant Enterobacteriaceae has raised significant concerns for both human and animal health, potentially compromising this last-resort antibiotic's efficacy[22]. This emerging threat underscores the urgent need for antimicrobial stewardship and new treatment strategies.

Data on the prevalence of antimicrobial resistance is limited in Africa. In 2014, the World Health Organization (WHO) reported that antimicrobial resistance surveillance in Africa is particularly difficult due to a lack of reliable medical data and laboratory capacity[23]. Wrong healthcare practices, lax health systems and irresponsible behaviour by citizens all contribute to the challenges of antimicrobial resistance.

Social research is needed to understand the neighbourhood context and complexity of human behaviour that contribute to antimicrobial resistance. This study sought to assess the prevalence of colistin-resistant bacteria in water sources in the Wa Municipality of the Upper West Region of Ghana.

MATERIALS AND METHODS

Study design: This study followed a cross-sectional and experimental design pattern to assess the prevalence of colistin-resistant bacteria from three water sources; boreholes, wells and tap water in the selected communities within the Wa Municipality. The study was carried out from May, 2023 to July, 2023.

Sampling technique: The simple random sampling technique was used for communities with a large population while the stratified random sampling technique was used for communities with large populations.

Study site: Samples were obtained from the immediate environs of Dr. Hilla Limann Technical University (DHLTU), specifically Kpongu, Sawaba, Chokor and Bamahu, all within the Wa Municipality. The municipality is located in the Upper West Region of Ghana and shares boundaries to the North with Nadowli District, to the Southwest with Wa West District and the East with Wa East District.

Study size and population: A total of 40 samples were collected from three different water sources; 16 samples from tap water, 16 samples from borehole water and 8 samples from well water spread across the selected communities.

Laboratory process: Samples, collected in sterile bottles, were promptly transported and analysed within 2 hrs. Water samples underwent filtration with a 0.22 µm syringe filter; the filter paper was placed in Brain Heart Infusion (BHI) broth and incubated at 35±°C for 18-24 hrs. The BHI cultures were sub-cultured onto MacConkey and Salmonella Shigella agar for general and selective isolation, respectively. Standard biochemical tests were used to identify isolates[24] while Kirby-Bauer Disk diffusion and micro broth dilution techniques were used to assess colistin susceptibility. Colistin (10 µg) disks were placed on Mueller Hinton agar for colistin resistance screening according to Kirby-Bauer disk-diffusion method following standard laboratory protocols[25]. Antimicrobial susceptibility was interpreted using EUCAST standards. For microbroth dilution, BMD panels with colistin sulphate were prepared. Four Mueller-Hinton broths with varying colistin disks (0, 1, 2 and 4 µg/mL) were incubated and bacterial isolates were added[26,27]. The MIC values were visually read after 16-20 hrs at 35°C. Results were read after 18-24 hrs; growth was confirmed by turbidity. The test was valid if growth occurred in the control and no "skipped well" was observed.

Statistical analysis: Data was summarized, coded and merged in Microsoft Excel 2021, with the water samples as the unit of analysis, then imported into Statistical Package for Social Scientists (SPSS) version 28 set at a 95% confidence interval and a p-value of 0.05 as statistically significant for analysis of results using frequencies and proportions and presented in tables and charts.

RESULTS AND DISCUSSION

Community distribution and demographics of collected samples: The 40 water samples collected originated from 4 communities in the Wa Municipality, including Bamahu, Chokor, Kpongu and Sawaba. More samples were gathered from the higher population areas of Sawaba and Kpongu. The majority of samples (74.6%) came from urban settlements, compared to only 25.4% from rural settlements. This focus on communities near the regional capital is relevant, as higher population density and infrastructure access in urban areas impact water quality through direct wastewater discharge, pollutant runoff and overuse of antimicrobials[28].

The 40 samples spanned three types of water sources used locally for drinking, washing and irrigation: Boreholes, taps and wells. Each source is contaminated by different microbes from human, animal, or environmental contact. Comparing results by source can reveal if certain points show higher resistance. Earlier studies revealed that borehole and well water sources contained higher bacteria contaminants compared to pipe-borne water[29–31]. Boreholes accessing vulnerable groundwater aquifers are also more contamination-prone than piped municipal supplies[28].

Prevalence and distribution of organisms isolated: Culture-based methods isolated a diversity of Gram-negative enteric organisms including *Citrobacter* species (28.5%), *Klebsiella* species (25%), *Serratia marcescens* (21.4%), *Proteus vulgaris* (14.3%), *Enterobacter* species (3.57%), *Providencia rettgeri* (3.57%) and *Salmonella* species (3.57%) from the water samples. Many clinically relevant drug-resistant genera were present, indicating widespread faecal contamination of water supplies from human and animal waste containing gut flora. Isolated organisms were distributed across the 4 communities without marked geographic predilections. However, some demographic associations emerged. *Klebsiella* species predominated among urban samples, while *Citrobacter* species dominated in rural settings (Fig. 1).

Phenotypic resistance testing methods and findings: Phenotypic colistin resistance was evaluated using both Kirby Bauer disk diffusion (KBDD) and broth microdilution minimum inhibitory concentration (MIC) quantification. The KBDD method indicated only 4.7% of isolates as resistant, while the MIC testing found 44.8% of isolates as resistant. This discrepancy highlights the superior sensitivity of MIC for detecting colistin resistance described in some earlier studies[32,33]. However, substantial resistance identified by both methods among community water samples without recent clinical antimicrobial pressure is alarming.

The MIC testing revealed resistant organisms occurring across all 4 communities (Fig. 2). In contrast, KBDD detected limited resistance, with single isolates in Chokor and Bamahu. Widespread resistance by MIC suggests colistin-resistant bacteria are prevalent through water sources in the Wa Municipality due to common antimicrobial contamination pressures and resistance gene exchange between organisms[19]. However, KBDD may still provide useful preliminary resistance screening where resources are constrained.

Statistical analysis also found a significant positive correlation between urban settlements and resistance detected via MIC testing (p = 0.049), indicating human population activities as likely resistance drivers. No other clear correlations between community, source or method were identified (Table 1).

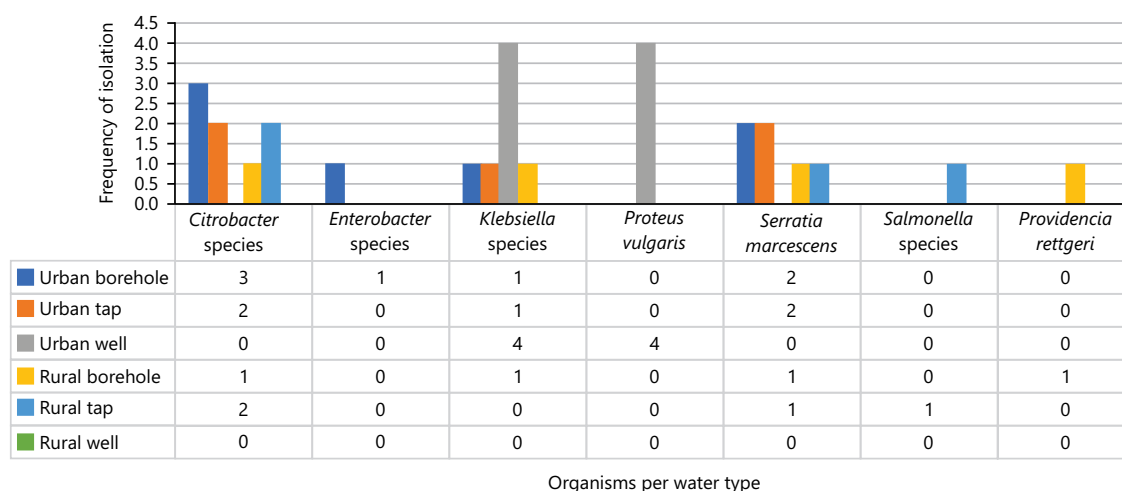|	*Citrobacter* species	*Enterobacter* species	*Klebsiella* species	*Proteus vulgaris*	*Serratia marcescens*	*Salmonella* species	*Providencia rettgeri*
Urban borehole	3	1	1	0	2	0	0
Urban tap	2	0	1	0	2	0	0
Urban well	0	0	4	4	0	0	0
Rural borehole	1	0	1	0	1	0	1
Rural tap	2	0	0	0	1	1	0
Rural well	0	0	0	0	0	0	0

Organisms per water type

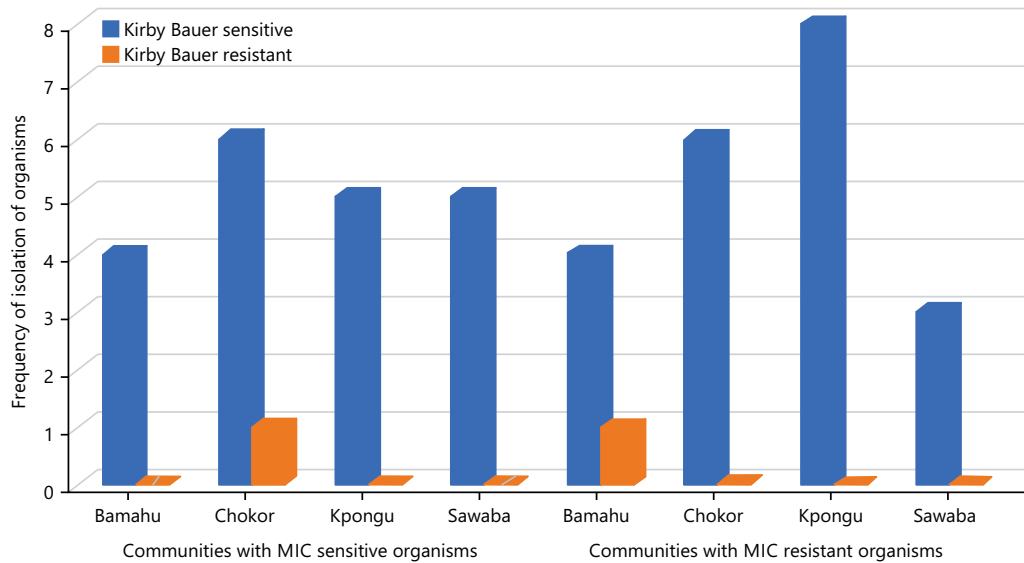Fig. 1: Distribution of microbes across urban and rural settings

Fig. 2: Resistance across communities and testing methods

Table 1: Correlation between community, settlement, water type, disk diffusion inhibition zone and MIC status

		Community	Settlement	Water type	Disc diffusion zone	Mic status
Community	Pearson correlation	1	0.165	0.122	0.017	-0.172
	Sig. (2-tailed)		0.211	0.358	0.913	0.196
	N	59	59	59	43	58
Settlement	Pearson correlation	0.165	1	-0.268*	0.243	0.259*
	Sig. (2-tailed)	0.211		0.040	0.117	0.049
	N	59	59	59	43	58
Water type	Pearson correlation	0.122	-0.268*	1	-0.209	-0.033
	Sig. (2-tailed)	0.358	0.040		0.178	0.805
	N	59	59	59	43	58
Disc diffusion zone	Pearson correlation	0.017	0.243	-0.209	1	0.103
	Sig. (2-tailed)	0.913	0.117	0.178		0.511
	N	43	43	43	43	43
Mic status	Pearson correlation	-0.172	0.259*	-0.033	0.103	1
	Sig. (2-tailed)	0.196	0.049	0.805	0.511	
	N	58	58	58	43	58

*Correlation is significant at the 0.05 level (2-tailed)

Microbiological findings by source and location: Among urban samples, numerically more resistant organisms originated from borehole water than the others (Fig. 1). This trend aligned with prior studies finding groundwater supplies more vulnerable to contaminant infiltration[28]. Within rural areas, the diversity of flora appeared lower but more organisms were isolated from boreholes than taps or wells (Fig. 1).

Breaking down results by community, variability emerged in the organism profiles and resistance levels across different sources:

- **Boreholes (Fig. 3):** More *Klebsiella* species in Kpongu/Chokor; more *Serratia marcescens* in Bamahu/Sawaba. This indicates potential small-scale differences in contaminant sources and selective pressures within locations
- **Taps (Fig. 3):** *Salmonella* species uniquely found in Chokor's piped groundwater indicates alarming fecal contamination which is possibly due to breaches in distribution system integrity or contaminated aquifers possible

- **Wells (Fig. 3):** Numerically more *Klebsiella* species and *Proteus vulgaris* were detected in Chokor/Kpongu wells. This suggests possible groundwater contamination by surface runoff, aquifer leakage, or direct fouling. Well depth and construction quality are risks

Together, these points to urbanization and reliance on vulnerable groundwater as likely increasing exposure to resistant bacteria through drinking, domestic and agricultural water use and resistance mechanisms continue to spread through all water sources.

Susceptibility profiles of isolated organisms: Analyzing susceptibility against source, location and organism variables also revealed informative trends: More diverse resistant organisms were found in urban compared to rural areas (8 types against 5 types) (Table 2). The only rural resistance was detected in *Klebsiella* species which showed intermediate resistance to colistin. Urban locations haboured additional resistant organisms (*Klebsiella*, *Proteus*, *Serratia* and *Citrobacter* species) primarily in boreholes and wells; indicating human impacts on resistance. By MIC testing, resistant phenotypes were detected in all 4 communities. Highest resistance prevalence was in Kpongu (*Citrobacter* and *Serratia* species) and Chokor (*Klebsiella* species and *Proteus* species). Lowest was in Bamahu (*Klebsiella* species). This reflects spatial heterogeneity between closely situated locations.

The MIC also identified resistant organisms (*Klebsiella* species, *Citrobacter* species and *Salmonella* species) in rural areas, whereas KBDD found none (Table 2). This underscores MIC's superior sensitivity. Resistance spanned all water sources (boreholes, taps, wells) and organisms (*Klebsiella* species, *Proteus* species and *Serratia* species).

This study revealed high levels of colistin-resistant Gram-negative bacteria contaminating drinking water sources in the Wa Municipality of Ghana. Nearly half of all isolates tested exhibited phenotypic colistin-resistance based on the sensitive broth microdilution minimum inhibitory concentration testing method. Resistant organisms were diverse, spanning *Klebsiella*, *Citrobacter*, *Salmonella*, *Serratia species* and other clinically relevant genera that are common gut flora.

Statistical analysis identified a significant association between urban settlements and colistin-resistance. Numerically more resistant organisms originated from boreholes than the other sources, especially in urban areas, reflecting vulnerabilities in accessed groundwater. There was also evidence of small-scale spatial heterogeneity in contamination and resistance profiles between closely situated communities.

Overall, findings likely reflect widespread faecal pollution from human and animal waste introducing gut flora and resistance genes into water systems. Unregulated antimicrobial use and poor waste management appear to account for resistance. The high prevalence of colistin-resistant enteric bacteria in drinking water presents a major public health risk in the Wa Municipality.

Improving sanitation infrastructure, water treatment and hygiene practices is critical to reducing faecal pollution from human and animal waste introducing resistant gut flora into water systems. Regulating antimicrobial use through stewardship programs and prescriber/consumer education is also essential to curb selection pressure driving resistance. Ongoing surveillance utilizing sensitive MIC testing should monitor resistance trends and identify vulnerable points in water systems requiring priority remediation, whether boreholes tapping contaminated aquifers or aging pipe distribution networks. A coordinated "One Health" approach across public health, veterinary, environmental and local governance sectors is necessary to control the selection and spread of antimicrobial resistance in this region.

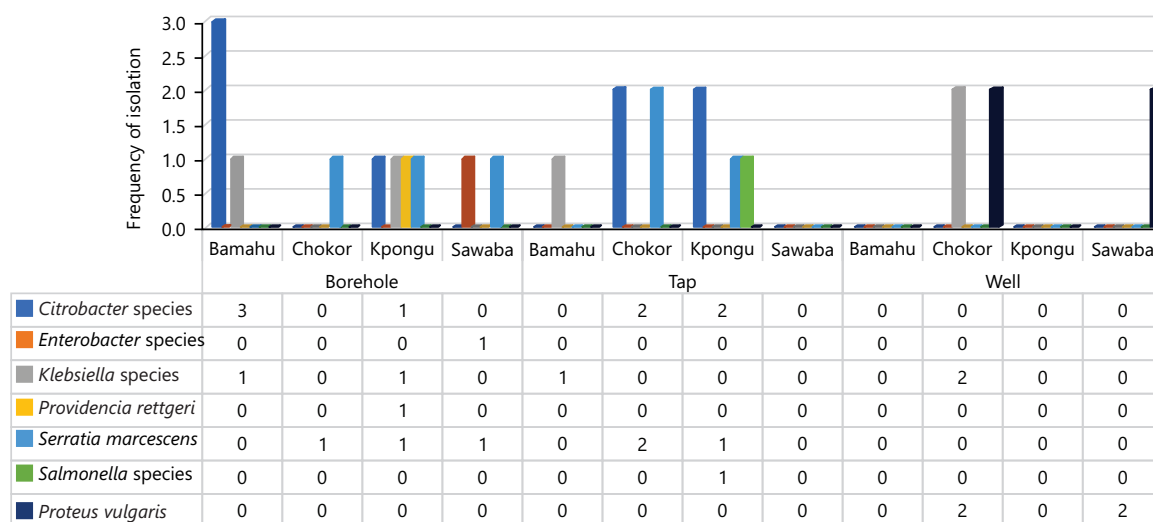|	Borehole				Tap				Well			
	Bamahu	Chokor	Kpongu	Sawaba	Bamahu	Chokor	Kpongu	Sawaba	Bamahu	Chokor	Kpongu	Sawaba
Citrobacter species	3	0	1	0	0	2	2	0	0	0	0	0
Enterobacter species	0	0	0	1	0	0	0	0	0	0	0	0
Klebsiella species	1	0	1	0	1	0	0	0	0	2	0	0
Providencia rettgeri	0	0	1	0	0	0	0	0	0	0	0	0
Serratia marcescens	0	1	1	1	0	2	1	0	0	0	0	0
Salmonella species	0	0	0	0	0	0	1	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	0	0	0	2	0	2

Fig. 3: Organisms isolated per community and water type

Table 2: Cross-tabulation of organisms isolated, resistance testing methods and settlement type

Settlement	Organism isolated	Disc diffusion status		MIC status	
		Sensitive	Resistance	Sensitive	Resistance
Urban	*Citrobacter* species	5	0	2	3
	Enterobacter species	1	0	0	1
	Klebsiella species	3	1	2	4
	Proteus vulgaris	2	0	3	1
	Serratia marcescens	4	0	2	2
Rural	*Citrobacter* species	3	0	1	2
	Klebsiella species	1	0	0	1
	Providencia rettgeri	1	0	1	0
	Serratia marcescens	2	0	1	1
	Salmonella species	3	0	0	1

CONCLUSION

This study reveals high levels of colistin-resistant bacteria in Wa Municipality's drinking water sources, posing a significant public health risk. Urban areas and boreholes showed higher resistance rates, indicating human activity impacts. Addressing this issue requires improving sanitation, water treatment, hygiene practices and antimicrobial stewardship. Immediate action and further research are crucial to combat resistance spread, safeguard community health and preserve antibiotic efficacy for future generations.

SIGNIFICANCE STATEMENT

This study addresses the critical gap in understanding antimicrobial resistance in water sources in Ghana, focusing on colistin resistance. The research is significant as it reveals high levels of colistin-resistant Gram-negative bacteria in drinking water sources in the Wa Municipality, with 44.8% of isolates showing resistance. The findings highlight the potential public health risk and the need for improved water management and antimicrobial stewardship. The study uniquely demonstrates the superiority of MIC testing over disk diffusion for detecting colistin resistance and identifies a significant association between urban settlements and resistance. These results contribute valuable data to inform public health strategies in Ghana and similar settings, emphasizing the importance of a One Health approach in combating antimicrobial resistance.

ACKNOWLEDGMENT

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