EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

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ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 16 No. 2 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.16 (2) pp.95- 109 (2024)*
DOI: 10.21608/EAJBSG.2024.379065

Egypt. Acad. J. Biolog. Sci., 16(2):95-109 (2024)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Detection of *PhoP*-mediated Colistin Resistance in Gram-negative bacteria without *mcr* genes in Water in the Ho Municipality, Ghana

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ARTICLE INFO

Article History
Received:4/8/2024
Accepted:10/9//2024
Available:14/9/2024

***Keywords*:**
Antibiotic resistance, colistin, *PhoP*, Gram-negative bacteria, water sources

ABSTRACT

Background: Colistin resistance in Gram-negative bacteria poses a significant threat to public health. This study investigated the prevalence of colistin resistance mediated by the *PhoP gene* in Gram-negative bacteria isolated from various water sources in the Ho Municipality, Ghana. **Methods:** Water samples were collected from different sources including boreholes, tap water, stored water, rainwater, streams, and wells. Gram-negative bacteria were isolated and identified using standard microbiological techniques and whole-genome sequencing. Colistin susceptibility was determined by broth microdilution. The presence of *mcr* genes was assessed by PCR, while *PhoP* was detected using nanopore sequencing. **Results:** Out of 132 water samples, 105 (79.55%) yielded Gram-negative bacterial growth. Phenotypic colistin resistance was observed in 52.1% of isolates, with 62.3% of resistant isolates carrying the *PhoP* gene. No *mcr* genes were detected. The prevalence of *PhoP* varied significantly among water sources, with direct tap water, stored tap water and well water showing the highest rates. *Klebsiella* spp., *Proteus* spp. and *Pseudomonas aeruginosa* were the most common *PhoP*-positive isolates. **Conclusion:** The high prevalence of *PhoP*-mediated colistin resistance in diverse water sources highlights the need for improved water treatment and sanitation practices. This study emphasizes the importance of environmental surveillance in understanding and controlling the spread of antibiotic resistance.

INTRODUCTION

Antibiotic resistance is a major global health challenge, with the increasing prevalence of multidrug-resistant Gram-negative bacteria posing a significant threat to public health (World Health Organization, 2017).

Colistin, a last-resort antibiotic, has been increasingly used to treat infections caused by multidrug-resistant Gram-negative bacteria (Falagas & Kasiakou, 2005). However, the emergence of colistin resistance, mediated by both plasmid-borne (*mcr*) and chromosomal (*PmrAB*, *PhoP-Pho*Q) mechanisms, has raised concerns about the future utility of this antibiotic (Poirel *et al.,* 2017).

The genomic basis of colistin resistance is complex and involves multiple mechanisms. Chromosomal mutations in genes such as *mgrB*, *pmrAB*, and *PhoP-PhoQ* can lead to modifications of the lipopolysaccharide (LPS) structure, reducing the binding affinity of colistin to bacterial cell membranes (Olaitan *et al.,* 2014). These modifications typically involve the addition of positively charged groups, such as phosphoethanolamine or 4-amino-4-deoxy-L-arabinose, to the lipid A portion of LPS, thereby reducing the net negative charge of the bacterial outer membrane and decreasing colistin binding (Jeannot *et al.,* 2017).

In recent years, the discovery of plasmid-mediated colistin resistance genes, particularly the *mcr* family, has significantly altered our understanding of colistin resistance transmission. The first *mcr* gene, *mcr*-1, was identified in 2015 in *Escherichia coli* isolates from animals and humans in China (Liu *et al.,* 2016). Since then, multiple variants (*mcr*-1 *to mcr*-10) have been reported globally in various bacterial species, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella enterica* (S. Wang & Shen, 2020). The *mcr* genes encode phosphoethanolamine transferases that modify the lipid A component of LPS, conferring resistance to colistin. The plasmid-mediated nature of these genes facilitates their rapid spread through horizontal gene transfer, posing a significant challenge to public health and raising concerns about the potential for widespread dissemination of colistin resistance (McGann *et al.,* 2016).

The *PhoP-PhoQ* two-component system is a key regulator of colistin resistance in Gram-negative bacteria (Miller *et al.,* 2005). Mutations that constitutively activate the *PhoP-PhoQ* system can lead to modifications in the bacterial lipopolysaccharide (LPS), reducing the affinity for colistin and resulting in resistance (Olaitan *et al.,* 2014). While the role of *PhoP* in mediating colistin resistance has been studied in clinical isolates (Cannatelli *et al.,* 2014; Cheng *et al.,* 2015), its prevalence and significance in environmental reservoirs, particularly in water sources, remain underexplored.

Aquatic environments can serve as important reservoirs and transmission routes for antibiotic resistance determinants (Baquero *et al.,* 2008; Taylor *et al.,* 2011). In Ghana, access to safe drinking water remains a challenge, with a significant proportion of the population relying on untreated surface and groundwater sources (Machdar *et al.,* 2013; Stoler *et al.,* 2012). These water sources are prone to contamination with antibiotic-resistant bacteria (Alotaibi, 2023; Cabral, 2010), including those harbouring colistin resistance genes(Mondal *et al.,* 2024). However, data on the prevalence and distribution of colistin resistance in aquatic environments in Ghana is currently limited.

This study aimed to address this knowledge gap by investigating the occurrence of colistin resistance among Gram-negative bacteria isolated from various water sources in the Ho Municipality of Ghana. The findings provide valuable insights into the role of aquatic environments in potentially harbouring and disseminating colistin resistance determinants, with implications

for public health and antibiotic stewardship efforts.

MATERIALS AND METHODS

Study Design, Study Area, Sample Distribution and Collection:

From June 2021 through December 2022, a cross-sectional investigation was undertaken in Ho Municipality, Ghana. 132 water samples were collected aseptically, each measuring 750mL, from an array of sources across multiple sites (Fig. 1). These included 27 boreholes, 45 direct tap connections, 30 stored tap water containers, 7 rainwater collections, 5 streams, and 18 wells. Well water extraction involved a meticulous process: a sterilized container was fastened to a rope and gently lowered into the well, avoiding contact with its sides, before being submerged and withdrawn once full. For tap water, a two-minute purge was performed to flush out stagnant water prior to collection. In the case of stored tap water, the container was first shaken, and then the sampling bottle was carefully immersed and filled underwater before being sealed and extracted. For boreholes, sampling was initiated with a two-minute flush to ensure freshwater flow. Stream water was collected by wading upstream and dipping containers below the water surface to capture physically uncontaminated samples. Rainwater collection employed a more passive approach: sterile, broad-necked bottles were strategically placed on elevated platforms, safeguarding against potential runoff contamination while allowing direct capture of falling precipitation. Upon collection, all samples were immediately sealed, labelled, and transported in ice chests to maintain a cool temperature pending laboratory examination. Each source was sampled in duplicate to ensure reliability.

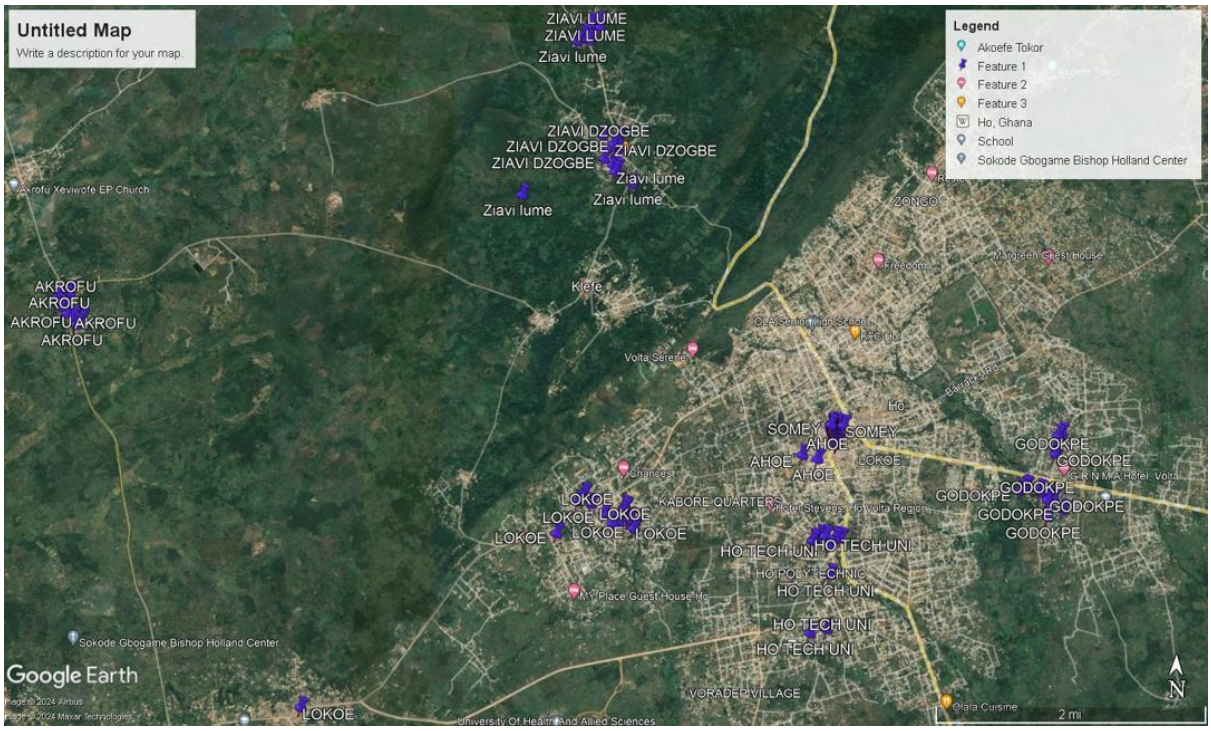

Fig. 1: Geospatial distribution of water samples collected.

Isolation and Identification of Gram-negative Bacteria:

200 mL of water samples were filtered through 0.22 µm membrane filters (Millipore). The filters were removed from their casing and placed in microcentrifuge tubes with peptone water. The tubes were vortexed to dislodge microbes from the filters and then the suspension was used to inoculate MacConkey agar plates. The

plates were incubated at 37^0C for 18-24 hours. Presumptive Gram-negative colonies were sub-cultured on nutrient agar and identified using standard biochemical tests (Mahon and Lehman, 2022). The organisms isolated were inoculated into 80% glycerol and kept in a -80^0C freezer for subsequent analysis. Further confirmation was performed by whole-genome sequencing (Lomonaco *et al.*, 2018; Luo *et al.*, 2017) using the MinION platform (Oxford Nanopore Technologies).

Phenotypic Colistin Susceptibility Testing:

Colistin susceptibility was determined by broth microdilution using colistin sulphate (Sigma-Aldrich) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). A stock solution of colistin sulphate (Sigma-Aldrich) was prepared at 5120 µg/mL in sterile distilled water. This solution was filter-sterilized using a 0.22 µm filter, then aliquoted and stored at -20^0C. From this stock, a working solution of 256 µg/mL was prepared by diluting in cation-adjusted Mueller-Hinton broth (CA-MHB). Two-fold serial dilutions were performed in CA-MHB to obtain final concentrations ranging from 0.125 to 64 µg/mL.

For inoculum preparation, a 0.5 McFarland standard suspension of the test organism was created in sterile saline. This suspension was diluted 1:150 in CA-MHB to achieve a final concentration of approximately 5 x 10^5 CFU/mL.

The broth microdilution method was performed using 96-well microtiter plates. 100 µL of each colistin dilution was added to the wells in columns 1-10. A growth control was included in column 11 with 100 µL of CA-MHB without colistin, and a sterility control in column 12 with 200 µL of CA-MHB. 100 µL of the prepared bacterial inoculum was added to wells in columns 1-11. The final colistin concentration range was 0.0625 to 32 µg/mL.

The plates were incubated at 35 ± 2^0C for 18-24 hours in ambient air. After incubation, the wells were observed for visible bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of colistin that completely inhibited visible growth. Isolates with MIC ≤2 µg/mL were considered susceptible and those with MIC >2 µg/mL were considered resistant, according to CLSI breakpoints (CLSI, 2021)

For quality control, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa ATCC* 27853 were included as control strains in each testing batch. The acceptable MIC range for *E. coli* ATCC 25922 was 0.25-2 µg/mL, and for *P. aeruginosa* ATCC 27853 was 0.5-4 µg/mL. MIC values and the interpretation (susceptible or resistant) were reported based on the CLSI breakpoints.

Detection of Colistin Resistance Gene in Isolates by Polymerase Chain Reaction:

The stored isolates were removed from the freezer, and the surface was scraped aseptically and inoculated into two separate 10 mL volumes of Brain Heart Infusion broths (Oxoid, UK). This was then incubated overnight in a shaking incubator (Gesellschataft fur Labortechnick mbH, Germany). The genomic DNA was extracted using an LBP Nucleic acid extraction and purification kit (Guangzhou LBP Medical Modified Science and Technology Co.Ltd). DNA concentration was assessed with the high Sensitivity dsDNA assay on the Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) using the manufacturer's protocol and quality was determined using the Nanodrop One Spectrophotometer (Thermo Fisher Scientific, USA). After extraction of DNA into microcentrifuge tubes and quantification of DNA concentration, the DNA was stored in cryo-boxes in a -20^0C freezer for PCR and whole genome sequencing. To allow fast and simultaneous detection of *mcr-1 to mcr-5* and *mcr-6* to *mcr-9* genes, multiplex PCR protocols as

published by (Rebelo *et al.*, 2018) and (Borowiak *et al.*, 2020) respectively, were employed.

Polymerase chain reaction (PCR) was employed to detect the presence of *mcr* genes (*mcr-1* to *mcr-10*) in phenotypically colistin-resistant isolates. The PCR master mix was prepared using one taq quick load 2x master mix with standard buffer (New England Biolabs®). Primers were reconstituted with nuclease-free water to create a 100 µM stock solution, then diluted to a 10 µM working solution. The PCR reaction mixture was prepared according to the manufacturer's instructions, with 2 µL of bacteria DNA added to each reaction. Two master mixes containing five primer combinations were prepared for each DNA extracted.Thermocycling was performed using an Eppendorf thermocycler (Germany) with the following conditions for *mcr-1* to *mcr-5*: initial denaturation at 94^0C for 15 minutes; 30 cycles of denaturation at 94^0C for 30 seconds, annealing at 58^0C for 90 seconds, and extension at 72^0C for 1 minute/kb; final extension at 72^0C for 10 minutes; and a final hold at 4^0C for 10 minutes while *mcr-6* to *mcr-10* had the following conditions: initial denaturation at 95^0C for 3 minutes; 30 cycles of denaturation at 95^0C for 30 seconds, annealing at 55^0C for 90 seconds, and extension at 72^0C for 1 minute/kb; final extension at 72^0C for 10 minutes; and a final hold at 4^0C for 10 minutes. The annealing temperature for some primers was slightly adjusted (52^0C for *mcr-6*, 50^0C for *mcr-7* and 53^0C for *mcr-8*) based on the manufacturer's instructions during the setup process. This was achieved using gradient PCR. Positive controls for *mcr* genes were obtained using in-house generated strains of *E. coli* (Deku *et al.*, 2022). The primer sequences were obtained from previously published studies, as referenced in Table 1. Details of the primer sequences and their corresponding product sizes, along with the source studies for each primer pair are presented in Table 1.

Table 1: Primers for multiplex-PCR

Primer name	Sequence (5'-3')	Target gene	Size (bp)	Reference
*mcr-1*_320bp_fw	AGTCCGTTTGTTCTTGTGGC	*mcr*-1	320	(Rebelo et al., 2018)
*mcr-1*_320bp_rev	AGATCCTTGGTCTCGGCTTG	*mcr*-1	320	(Rebelo et al., 2018)
*mcr-2*_700bp_fw	CAAGTGTGTTGGTCGCAGTT	*mcr*-2	715	(Rebelo et al., 2018)
*mcr-2*_700bp_rev	TCTAGCCCGACAAGCATACC	*mcr*-2	715	(Rebelo et al., 2018)
*mcr-3*_900bp_fw	AAATAAAAATTGTTCCGCTTATG	*mcr*-3	929	(Rebelo et al., 2018)
*mcr-3*_900bp_rev	AATGGAGATCCCCGTTTTT	*mcr*-3	929	(Rebelo et al., 2018)
*mcr- 4*_1100bp_fw	TCACTTTCATCACTGCGTTG	*mcr*-4	1,116	(Rebelo et al., 2018)
*mcr-4*_1100bp_rev	TTGGTCCATGACTACCAATG	*mcr*-4	1,116	(Rebelo et al., 2018)
*mcr-5*_fw	ATGCGGTTGTCTGCATTTATC	*mcr*-5	1,644	(Rebelo et al., 2018)
*mcr-5*_rev	TCATTGTGGTTGTCCTTTTCTG	*mcr*-5	1,644	(Rebelo et al., 2018)
Mcr-6_mp_fw	AGCTATGTCAATCCCGTGAT	*mcr*-6	252	(Borowiak et al., 2020)
Mcr-6_mp_rev	ATTGGCTAGGTTGTCAATC	*mcr*-6	252	(Borowiak et al., 2020)
Mcr-7_mp_fw	GCCCTTCTTTTCGTTGTT	*mcr*-7	551	(Borowiak et al., 2020)
Mcr-7_mp_ rev	GGTTGGTCTCTTTCTCGT	*mcr*-7	551	(Borowiak et al., 2020)
Mcr-8_mp_fw	TCAACAATTCTACAAAGCGTG	*mcr*-8	856	(Borowiak et al., 2020)
Mcr-8_mp_ rev	AATGCTGCGCGAATGAAG	*mcr*-8	856	(Borowiak et al., 2020)
Mcr-9_mp_fw	TTCCCTTTGTTCTGGTTG	*mcr*-9	1011	(Borowiak et al., 2020)
Mcr-9_mp_ rev	GCAGGTAATAAGTCGGTC	*mcr*-9	1011	(Borowiak et al., 2020)
mcr-10_fw (090065_up_SacI)	AAAAAAGAGCTCTCCGCTTTGTATCCCAATAC	*mcr*-10	1620	(C. Wang et al., 2020)
*mcr-10*_rev (090065_down_EcoRI)	AAAAAAGAATTCTTTTATAATTTCCGGCAGCA	*mcr*-10	1620	(C. Wang et al., 2020)

PCR products were analysed by gel electrophoresis to identify the presence of specific *mcr* genes based on the expected product sizes. Positive and negative controls, obtained from the laboratory's strain collection, were included in each PCR run to ensure the reliability of the results.

Detection of Colistin Resistance Gene by Nanopore Sequencing:

Genomic DNA was extracted from colistin-resistant isolates using the LBP Nucleic acid extraction and purification kit (Guangzhou LBP Medical Modified Science and Technology Co.Ltd). DNA concentration was assessed with the high Sensitivity dsDNA assay on the Qubit 4 Fluorometer (Thermo Fisher Scientific, USA) using the manufacturer's protocol and quality was determined using the Nanodrop One Spectrophotometer (Thermo Fisher Scientific, USA). Sequencing libraries were prepared using the Rapid Barcoding Kit 96 (SQK-RBK110.96) and sequenced using Spot-ON Flow Cell (FLO-MIN106D R9.4.1 Version) and the MinION Mk1C device (Oxford Nanopore Technologies, UK).

The generated reads were stored in the form of fast5 formats which were converted into fastq reads using the guppy basecaller package and the backbone script below:

guppy_basecaller -i fast5 -s fastq --flowcell FLO-MIN106 --kit SQK-RBK110-96 -x cuda:all

where -i denotes the full path to the directory containing the input fast5 files.

A demultiplexing step was then run to separate the various fastq reads into their respective barcodes using the guppy barcoder package, all part of the guppy suite using the backbone script below:

guppy_barcoder -i pass -s barcodes --barcode_kits SQK-RBK110-96 - x cuda:all

where -i will be the full path to the "pass" directory containing your basecalled reads.

Preliminary detection of the colistin resistance gene was determined using the EPI2ME cloud-based analysis platform (Metrichor Ltd., UK) via the EPI2ME desktop agent application software (v.18.01.6). Two separate pipelines with pre-configured workflows were run using the default parameters: FASTQ WIMP (What's In My Pot) for the rapid detection and quantification of species, and FASTQ Antimicrobial Resistance for the rapid AMR profiling. The "detect barcode" option was turned off for the analysis and results were sorted out into the respective folders in which they were uploaded, corresponding to the barcode names from the initial guppy run.

The presence of colistin resistance genes was confirmed using the Resistance Gene Identifier (RGI) tool and the Comprehensive Antibiotic Resistance Database (CARD) (Alcock *et al.,* 2020). Further polishing of reads was done using Porechop (v.0.2.3) following a concatenation of reads step using the following commands:

cat barcode*/*.fastq > barcode* merged. Fastq

Where the code above assumes the concatenation step is being operated from the respective barcode directory.

porechop -i /barcode*_merged.fastq -o barcode*_trimmed.fastq

Where -i is the directory containing your merged barcoded reads.

Select barcodes were sampled from which consensus sequences were constructed using Canu (v.1.7) using the backbone command:

canu -d /path/to/output_directory -p output_file_name genomeSize="4.8m" maxInput Coverage=10000 corOut Coverage=10000 corMhap Sensitivity= high corMinCoverage=0 redMemory=32 oeaMemory=32 batMemory=60 -nanopore /path/to/merged_input_reads

Parameters such as maxInputCoverage, corOutCoverage, corMhapSensitivity and corMinCoverage were set to their default parameters, whereas redMemory, oeaMemory, and

batMemory were set based on the computing power of the device the assembly was run on.

The generated contigs were run by BlastN programs for alignment calling and possible ARG identification in the NCBI nucleotide collection database. Detailed scripts used in post-sequencing analysis can be found in the supplementary sheet.

Data Analysis:

Data was analysed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarize the prevalence of colistin resistance and *PhoP* gene detection. The chi-square test and Fisher's exact test were used to compare the prevalence of colistin resistance genes across different water sources. A p-value <0.05 was considered statistically significant.

RESULTS

Out of the 132 water samples analysed, 105 (79.55%) yielded Gram-negative bacterial growth. The most common bacteria isolated belonged to *Klebsiella* spp. (43.1%), *Proteus* spp. (17%), *Citrobacter* spp. (11.4%), and *Salmonella* spp. (8.6%) (Fig, 2).

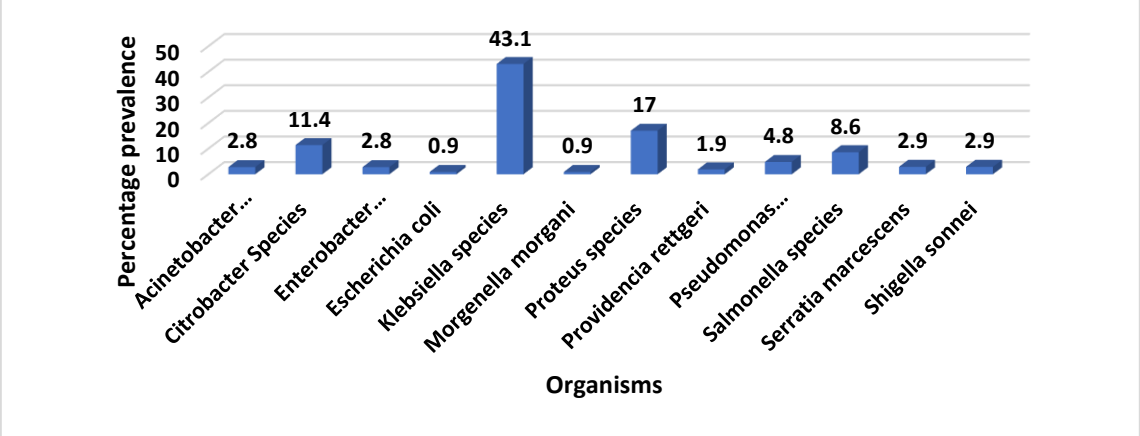

Fig. 2: Organisms identified via culture and biochemistry techniques

Colistin phenotypic susceptibility testing revealed that 52.1% of the Gram-negative isolates were resistant to colistin while nanopore sequencing detected the presence of the *PhoP* gene in 62.3% of the phenotypically resistant isolates (Fig. 3).

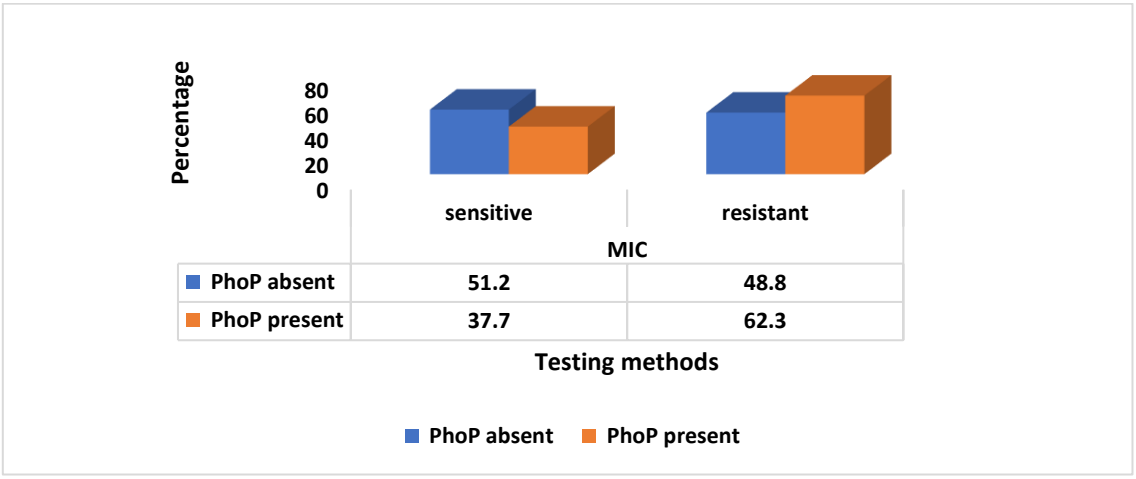

Fig. 3: Comparison between resistance detection methods

Although sampling proportions across the various water sources were not uniform, the prevalence of *PhoP* varied significantly among the different water sources ($p<0.05$). Direct tap water showed the highest diversity of colistin-resistant organisms with rainwater samples totally devoid of colistin-resistant organisms. *Escherichia coli* was found only in direct tap water while *Klebsiella pneumoniae* was detected in stored tap, stream, and well water. *Proteus mirabilis* was predominantly detected in stored tap water, with some presence in direct tap water while *Pseudomonas aeruginosa* was most widespread across the various water sources. The highest diversity of resistant species was observed in direct tap water, stored tap water and well water (Table 2).

Table 2: Percentage prevalence of colistin-resistant organisms per the various water sources

Organisms with *PhoP*	Borehole	Direct tap	Stored tap	Rain	Stream	Well
Escherichia coli	0	100	0	0	0	0
Klebsiella pneumoniae	0	0	25	0	25	50
Proteus mirabilis	0	20	80	0	0	0
Pseudomonas aeruginosa	18.1	27.3	27.3	0	0	27.3
Enterobacter roggenkampii	0	100	0	0	0	0
Acinetobacter baumannii	0	50	0	0	0	50
Acinetobacter soli	0	100	0	0	0	0
Serratia marcescens	0	50	0	0	50	0
Serratia nematodiphila	100	0	0	0	0	0
Providencia stuartii	0	0	0	0	0	100

Seasonal variation in colistin resistance profiles was studied using both the Minimum Inhibitory Concentration (MIC) method and the Whole Genome Sequencing (WGS) method across rainy and dry seasons (Fig. 4). The results suggest minimal differences in resistance profiles between the two seasons, indicating that the prevalence of colistin resistance is relatively stable throughout the year.

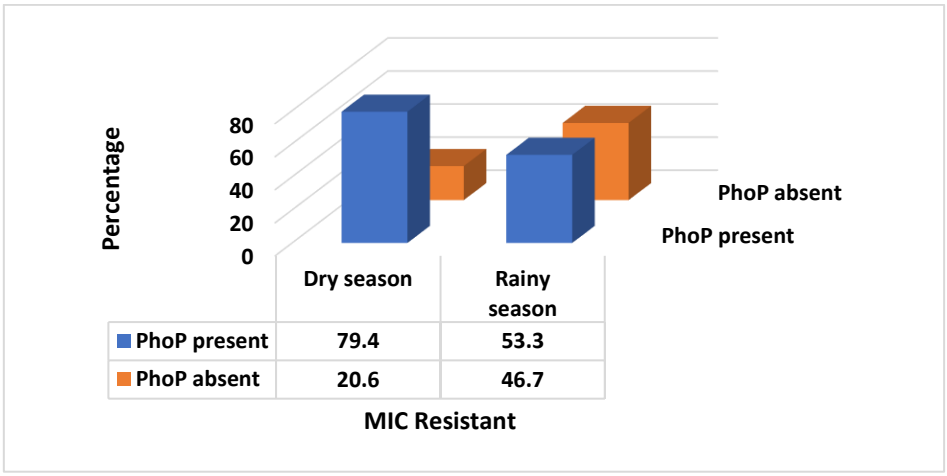

Fig. 4: Seasonal Differences in Resistance Profile.

DISCUSSION

This study investigated the prevalence of colistin resistance among Gram-negative bacteria isolated from various water sources in the Ho Municipality, Ghana. Colistin, a last-resort antibiotic, has been increasingly used to treat infections caused by multidrug-resistant Gram-negative bacteria (Falagas & Kasiakou, 2005). However, the emergence of colistin resistance, mediated by both plasmid-borne (*mcr*) and chromosomal (*PmrAB, PhoP*Q) mechanisms, has raised concerns about the future utility of this antibiotic (Poirel *et al.*, 2017).

In this study, Gram-negative bacteria were isolated and identified using standard biochemical tests and whole-genome sequencing. The most common bacteria species isolated were *Klebsiella*, *Proteus*, *Citrobacter*, and *Salmonella.* These findings are consistent with previous studies that have reported the presence of these Gram-negative bacteria in various water sources (Akpan *et al.*, 2020; Alabi & Fatoyinbo, 2016; Pandey *et al.*, 2014).

Phenotypic colistin susceptibility testing revealed a high prevalence of colistin resistance among Gram-negative isolates across the various water sources. This is similar to the findings of Osisiogu *et al.* (2023) that reported a high prevalence of 46% colistin resistance by Gram-negative organisms. This high prevalence of colistin resistance in aquatic environments is alarming, as it suggests that these water sources may serve as reservoirs for the dissemination of resistance determinants (Baquero et al., 2008; Taylor *et al.*, 2011), and poses a significant risk to public health, as these bacteria may colonize humans and animals, leading to the spread of resistance (Olaitan *et al.*, 2016).

Despite the high prevalence of phenotypic colistin resistance observed in this study, PCR targeting *mcr* genes (*mcr-1* to *mcr-10*) yielded negative results for all isolates. This unexpected finding prompted further investigation into the genetic basis of colistin resistance in these environmental isolates. To elucidate the underlying resistance mechanisms, we employed next-generation sequencing techniques. This approach allowed for a comprehensive analysis of the bacterial genomes, enabling the detection of alternative resistance determinants that may have been missed by targeted PCR assays. The absence of *mcr* genes in phenotypically resistant isolates emphasizes the complexity of colistin resistance mechanisms and highlights the potential for a novel or less common resistance determinants in environmental bacteria.

Nanopore sequencing thus detected the presence of the *PhoP* gene in 62.3% of the colistin-resistant isolates. The *PhoP-PhoQ* two-component system is a key regulator of colistin resistance in Gram-negative bacteria (Miller *et al.*, 2005). Mutations that constitutively activate the *PhoP-PhoQ* system can lead to modifications in the bacterial lipopolysaccharide (LPS), reducing the affinity for colistin and resulting in resistance (Olaitan *et al.*, 2014). The high prevalence of *PhoP* in colistin-resistant isolates suggests that this chromosomal resistance mechanism plays a significant role in the aquatic environment. Some other studies have reported lower occurrence (22%, 38.5%, and 14.16%) of colistin resistance compared to this present study (Elbediwi *et al.*, 2019; Jofré Bartholin *et al.*, 2023; Tabut P, 2020). However, all three studies focused on the detection of *mcr* genes, unlike this current study which detected *PhoP genes.* It is worth mentioning that not all phenotypically resistant isolates carried the *PhoP* gene in this present study (Figure 3), suggesting the potential presence of other resistance mechanisms. Also, some phenotypically sensitive isolates were found to possess *PhoP* genes. Several factors may account for this phenomenon; there may be other genes or factors that counteract or suppress

the effects of *PhoP*, maintaining colistin sensitivity even when the gene is present (Jeannot *et al.,* 2017). More so, mutations or regulatory changes could prevent the gene from being transcribed or translated into a functional protein (Olaitan *et al.,* 2014). The prevalence of genotypic colistin resistance was higher than the phenotypic colistin resistance reported in a similar study, suggesting that not all isolates carrying *mcr* genes expressed phenotypic resistance (Tabut P, 2020).

The prevalence of *PhoP* varied significantly among the different water sources in this study, thereby indicating that the type of water source plays a role in the presence and persistence of colistin-resistant bacteria carrying the *PhoP* gene. The highest rates of *PhoP* detection were observed in well water, borehole water, and direct tap water. This variation in *PhoP* prevalence may be attributed to differences in water quality, sanitation practices, and the presence of selective pressures in these environments (Agyare *et al.,* 2019; Baquero *et al.,* 2008). The high prevalence of *PhoP* in well water and borehole water is particularly alarming, as these sources are often used for drinking and domestic purposes in many developing countries, including Ghana where the main sources of water are wells, boreholes, taps and streams (Kanjin *et al.,* 2023). The high prevalence of *PhoP* in borehole water is also alarming, as boreholes are often considered a safer alternative to surface water sources. This could suggest that groundwater may also be a significant reservoir for antibiotic-resistant bacteria. Stored tap water had a lower rate of *PhoP* detection compared to direct tap water. This could potentially be due to the attenuation of bacterial populations during storage or differences in sampling and storage practices. The high prevalence of colistin-resistant bacteria in direct tap water on the other hand raises questions about the effectiveness of current water treatment processes in eliminating antibiotic-resistant bacteria.

Among the *PhoP*-positive isolates, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the most common species. This is similar to the findings of Elbediwi *et al.* (2019) that reported the presence of *Klebsiella pneumoniae*, *E. coli* and some other key pathogens. The species mentioned are important opportunistic pathogens associated with various nosocomial (hospital-acquired) and community-acquired infections (Paczosa & Mecsas, 2016; Pang *et al.,* 2019; Schaffer & Pearson, 2015). The high prevalence of *PhoP* in these species highlights the potential risk of colistin resistance dissemination in clinical settings. This suggests that healthcare environments must be vigilant in monitoring and controlling these pathogens to prevent the spread of resistance, which could significantly complicate treatment options for infections caused by multi-drug-resistant organisms.

Escherichia coli, another important pathogenic species, had a notable *PhoP* prevalence in this study which is quite higher than the prevalence (23%) reported by Elbediwi *et al.* (2019). *E. coli* is a common indicator of faecal contamination in water sources and is often used to assess the microbiological quality of water (Tallon *et al.,* 2005). The presence of *PhoP*-mediated colistin resistance in *E. coli* suggests that faecal contamination may contribute to the spread of resistance determinants in aquatic environments (Olaitan *et al.,* 2015).

The seasonal variation in resistance profiles was also investigated, with minimal differences observed between rainy and dry seasons. This finding suggests that the prevalence of colistin resistance by *PhoP* is relatively stable throughout the year, indicating a persistent problem in the aquatic environment. This however is in contrast with a study done in Thailand that found colistin resistance to appear differently per season and reported colistin resistance to be higher during the dry season than during the rainy season.

The authors attributed the differences in the temperature, pH, and electrical conductivity of the water as the possible reasons for the seasonal variation. They also noted that the dry season has less rainfall than the rainy season, thus reducing the flow of water in rivers and causing a greater accumulation of bacteria in the water sources than in the rainy season (Tabut P, 2020)

The widespread occurrence of *PhoP*-mediated colistin resistance in aquatic environments in the Ho Municipality, Ghana, emphasizes the need for improved water treatment and sanitation practices to limit the spread of resistance determinants. Access to safe drinking water remains a challenge in many developing countries, and the presence of antibiotic-resistant bacteria in water sources can have serious public health implications (Agyare *et al.,* 2019; Shannon, 2008).

Conclusion

This study provides valuable insights into the prevalence and distribution of *PhoP*-mediated colistin resistance among Gram-negative bacteria in various water sources in the Ho Municipality, Ghana. The high prevalence of colistin resistance and the presence of the *PhoP* gene in diverse bacterial species underscore the need for concerted efforts to monitor and control the spread of resistance in aquatic environments. Improved water treatment, sanitation practices, and public health interventions are crucial to mitigate the risk posed by antibiotic-resistant bacteria in water sources and to protect public health. The One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, should be adopted to tackle the problem of antibiotic resistance in a comprehensive manner

Declarations:

Ethical Approval: This study forms part of a larger study that received ethical clearance from the Committee on Human Research, Publications and Ethics of Kwame Nkrumah University of Science and Technology (KNUST)- Ghana, with reference number (CHRPE/AP/371/20).

Conflicts of Interest: We declare that the authors have no competing interests, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Authors Contributions: Emmanuel U. Osisiogu, Kwabena O. Duedu, Bhavana Singh and Patrick K. Feglo developed the concept and directed the research. Emmanuel U. Osisiogu and Kwabena O. Duedu carried out sample collection, laboratory and data analysis as well as manuscript draft preparation. All authors have read, reviewed, and approved the content of the last version of this manuscript.

Consent to publication: Not applicable.

Funding: This work was supported by the research facilities provided by Tractilis BioLabs and Duedu Laboratory. The authors have no additional funding sources to disclose.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements: We would like to express our deepest gratitude to Ms. Priscilla Essandoh, Mr. Hubert Agbogli, Emmanuel Nattah, Enyonam Monia Honyo, Sena Adegbedzi, Cyril Kumah, Emmanuel Ativi, Idan Banson, Rosina Carr, and Hayford Offei for their invaluable assistance and support in the laboratory. Their dedication and hard work in assisting in the performance of these extensive experimental procedures and analyses were instrumental to the success of this research.

We sincerely thank Dr. Jones Gyamfi, Dr. John Gameli Deku, and Mr. Emmanuel Allotey for generously providing their technical expertise and guidance throughout this research. Their wisdom and insights greatly facilitated the progression of the study.

We are extremely thankful to Tractilis BioLabs and the Duedu laboratory for granting us access to their state-of-the-

art facilities and equipment. This work would not have been possible without the resources and infrastructure they kindly provided.

Finally, we are grateful to the inhabitants of the communities where sampling was done. Their contributions are invaluable.

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