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RESEARCH ARTICLE

Antibiotic Resistance of Bacteria Isolated from Water Supplies Used in Poultry Production in Ashanti Region of Ghana

Frank B. Osei[1], Vivian E. Boamah[1], Yaw D. Boakye[1], Christian Agyare[1,*] and Robert C. Abaidoo[2]

[1]*Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Department of Theoretical and Applied Biology, Faculty of Bioscience, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

Abstract:

Objective:

Water plays an important role in both domestic and commercial settings. Pathogenic microbial contaminants, however, render water unsafe for use. There are several reports on the quality of water used for drinking purposes in humans but few studies have been conducted on the microbial quality of water used in animal farming.

Methods:

In this study, the resistance pattern of bacterial isolates from drinking water used in poultry production in the Ashanti region of Ghana from our previously published report was determined. The presence of *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and coagulase-negative *Staphylococci* was determined using selective culture media (pour plate method) and confirmed through Gram staining and biochemical reactions. Antibiotic sensitivity of isolates was determined followed by detection of Extended-Spectrum Beta-Lactamase (ESBL) producing Gram-negative isolates.

Results:

The study revealed that water used in poultry farms contains sources of multi-drug resistant strains of *E. coli, S. typhi, S. aureus* and coagulase-negative *staphylococci*. *E. coli, S. typhi, S. aureus* and coagulase-negative *staphylococci* were recovered from 31%, 36%, 64% and 19% of samples, respectively. Majority of these isolates were resistant to cephalosporins and penicillins. Almost 95% of the bacterial isolates were multi-drug resistant. None of *E. coli* and *S. typhi* isolates produced ESBL.

Conclusion:

There is a need for stringent regulations and stringent measures should be taken to make these various sources of water safe for use in animal husbandry as these waters are a potential source of pathogenic and resistant bacterial strains which can cause infections to the animals and farm workers.

Keywords: Antibiotic resistance, Water samples, Antibiotics, Bacterial isolates, Pathogenic bacterial poultry, *E. coli*, *S. typhi*, *S. aureus*.

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1. INTRODUCTION

Water is one of the most important and abundant resources on earth which may either be safe or unsafe because it can potentially harbour various infective agents [1]. It has been estimated that about 1.1 billion people in the world are unable to access safe drinking water [2]. A report by Aning [3] indicated that the poultry industry in Ghana is quite extensive with many Ghanaians producing poultry and poultry products at commercial levels. Majority of poultry farms in Ghana are distributed within the Ashanti, Brong-Ahafo and the Greater Accra regions of the country. Most of these poultry farms rely on groundwater (boreholes and wells) as their main source of water supply for the poultry birds and other poultry processing purposes on the farms [4]. Water also serves as a medium for numerous microorganisms, most of which are disease-causing [5]. The use of contaminated water in poultry farming processes may serve as a source of infection in the birds.

Infections in poultry can lead to low egg production, loss

* Address correspondence to this author at Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; Tel: 233246369803;
E-mail: cagyare.pharm@knust.edu.gh; chrisagyare@yahoo.com

of weight in poultry and death of poultry [6], and some of these infectious agents can also be transmitted from farm animals to farm workers [7]. According to Puzelli *et al.* [8], viral particles of avian H7 influenza viruses were identified in the sera of farm workers exposed to poultry birds infected with the viral particles in Italy. One way of preventing such occupational exposure of farm workers and others to zoonotic diseases is to control and prevent the diseases in the poultry [9]. Identification of the possible sources of infections will help to devise measures to control their transmission. Possible sources of infections in the poultry may include farm workers, water used to feed the birds, poultry feed, feeding equipment and poultry litter. Most of these infectious agents have been identified in the litter of poultry birds [4].

Ashanti region is one of the three regions in Ghana with the highest number of poultry farms, of which many farms rely on groundwater as their source of water [4]. Various pathogenic microbial organisms have been identified in groundwater (boreholes and wells) used as drinking water in various communities in the Ashanti region of Ghana [10]. Microbial assessment of water used in these farms will help control the rate of infections in the poultry birds. In our previous report, we identified bacterial isolates including *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and coagulase-negative *Staphylococci* in various sources of water used in some selected poultry farms in the Ashanti region of Ghana [11]. The emergence of microbial resistance to various antibiotics calls for prompt actions to determine the susceptibility of microbial agents found in the water used in the various poultry farms and possibly determine the mechanism of resistance in order to control the infections likely to be caused by these infectious agents to poultry birds as well as poultry farmers. In this study, the resistance pattern of the isolated bacterial strains including *E. coli, S. typhi, S. aureus* and coagulase-negative *staphylococci* isolates from drinking water samples used for poultry production in the Ashanti region of Ghana from our previous study [11] and the detection of Extended-Spectrum Beta-Lactamase (ESBL) producing organisms among the resistant bacterial isolates were deter-mined.

2. MATERIALS AND METHODS

All the chemicals and reagents were purchased from Sigma-Aldrich, London, UK, unless otherwise stated.

2.1. Selection of Farms

Purposive sampling technique was employed in the selection of the 100 poultry farms out of the total estimated number of 820 poultry farms in the Ashanti region of Ghana. Farms with 500 birds or more were used for the study.

2.2. Sampling of Water from Poultry Farms

Samples of water were picked according to the guidelines from WHO [12] and transported to the laboratory on ice.

2.3. Isolation and Identification of Bacterial Isolates from Water Samples

The isolation and identification of the various bacterial isolates from the samples collected were performed according to the methods described by Osei *et al*. [11]. One millilitre (1 mL) of water sample was aseptically inoculated into 10 mL freshly prepared nutrient broth (Thermo-Fisher, London, UK) and incubated at 37˚C for 24 h. Isolates of *Escherichia coli* were obtained by inoculating 1 mL of the 24 h sample broth culture into 20 mL freshly prepared sterile MacConkey agar (Thermo-Fisher, London, UK) and confirmed through Gram-staining and biochemical tests including catalase activity in 3% hydrogen peroxide, indole production test in tryptone water, citrate utilization test and Methyl Red-Voges Proskauer (MRVP) test [13]. Isolates of *Salmonella typhi* were obtained by inoculating 1 mL of the 24 h sample broth culture into 20 mL freshly prepared sterile bismuth sulphite agar and confirmed through Gram-staining and biochemical tests including hydrogen-sulphide production test in peptone water, indole production test in tryptone water, citrate utilization test and catalase activity in 3% hydrogen peroxide solution [13].

Isolates of *Staphylococcus aureus* were obtained by inoculating 1 mL of the 24 h sample broth culture into 20 mL freshly prepared sterile mannitol salt agar and confirmed through Gram-staining and biochemical tests inclu-ding catalase activity in 3% hydrogen peroxide solution, coagulase test and haemolysis test on blood agar [13]. Isolates of coagulase-negative *Staphylococci* were obtained by inoculating 1 mL of the 24 h sample broth culture into 20 mL freshly prepared sterile mannitol salt agar (Thermo-Fisher, London, UK) and confirmed through Gram-staining and biochemical tests including catalase activity in 3% hydrogen peroxide solution, coagulase test and haemolysis test on blood agar [13].

2.4. Antimicrobial Susceptibility Testing

The Kirby-Bauer disk plate method was employed following EUCAST 2017 guidelines to determine the susceptibility of isolates to selected reference antibiotics [14, 15]. Ten microlitres of 24 h broth culture of bacterial isolates of turbidity equivalent to a 0.5M MacFarland standard was aseptically spread on 20 mL of freshly prepared sterile Mueller-Hinton agar (Thermo-Fisher, London, UK). Antibiotic discs were then put on the surface of the Mueller-Hinton agar after drying for 10 min at room temperature of 25˚C prior to incubation at 37˚C for 24 h. The reference antibiotics used in the study were cephalexin-30 µg, cefoxitin-30 µg, cefotazime-30 µg, ceftriaxone-30 µg, cefta-zidime-30 µg, cefpodoxime-10 µg, cefpodoxime/clavulanic acid-11 µg, norfloxacin-10 µg, ciprofloxacin-5 µg, aztreonam-30 µg, tetracycline-30 µg, doxycycline-30 µg, erythromycin-15 µg, tobramycin-10 µg, gentamycin-10 µg, ampicillin-10 µg, chloramphenicol-30 µg and sulphamethoxazole/trimethoprim-25 µg. The average values for the zone of inhibition from three determinations were recorded and interpreted following EUCAST [14] breakpoints.

2.5. Detection of Extended-Spectrum Beta-Lactamase (ESBL) Enzymes in Enterobacterial Isolates

The double disk synergy method was employed [14, 16] using 20 mL of freshly prepared sterile Mueller-Hinton agar seeded with 10 µL of 24 h broth culture (0.5M MacFarland standard) of bacterial isolates and incubated at 37˚C for 24 h. The reference antibiotics used in the study included cef-

triaxone-30 µg, ceftazidime-30 µg, cefotaxime-30 µg, aztreonam-30 µg, cefpodoxime-30 µg and cefpodox- ime +clavulanic acid-11 µg. A difference of 5 mm or more in zone diameter between cefpodoxime and cefpodoxime + clavulanic acid indicated that the ESBL enzymes acted against the individual beta-lactam antibiotics but have been inhibited by clavulanic acid with the synergistic effect of cefpodoxime and clavulanic acid [14].

3. RESULTS

3.1. Detection of Bacterial Isolates

Sixty-four (64), 36, 31 and 19 different strains of *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and coagulase-negative *Staphylococcus,* respectively, were isolated from the water samples (Fig. **1**).

3.2. Antibiotic Resistance Profiles of Isolates

Thirty (96.77%) isolates of *E. coli* were resistant to ampicillin, cefoxitin, cephalothin and cephalexin. Twenty-nine (93.55%) *E. coli* isolates were resistant to cefpodoxime, with 24 (77.42%) *E. coli* isolates being resistant to sulpha-methoxazole/trimethoprim. Eight (25.81%) *E. coli* isolates showed resistance to cefotaxime, while 3 (9.68%) *E. coli* isolates were resistant to aztreonam. Two different strains (6.45%) of *E. coli* isolates were resistant to gentamicin and ceftriaxone. One (3.22%) *E. coli* isolate each showed resistance to ceftazidime and chloramphenicol. All the thirty-one (100%) *E. coli* isolates were susceptible to tobramycin, norfloxacin and ciprofloxacin (Fig. **2**).

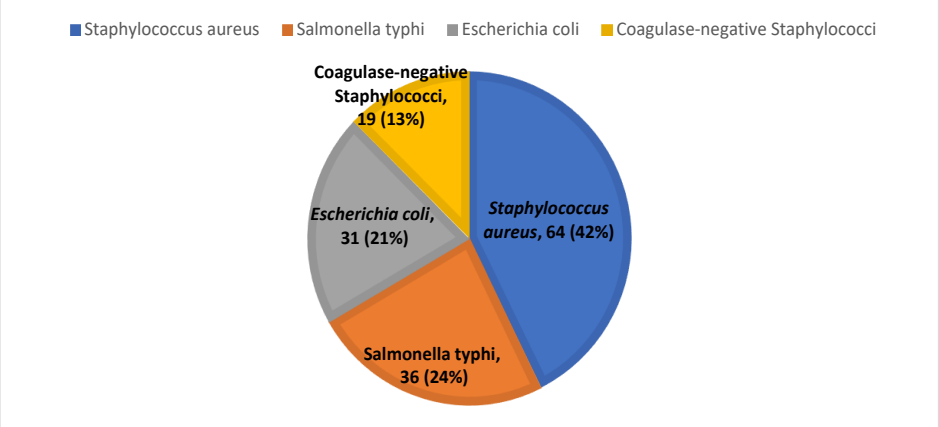

Fig. (1). Number of different bacterial strains isolated from the farm water samples.

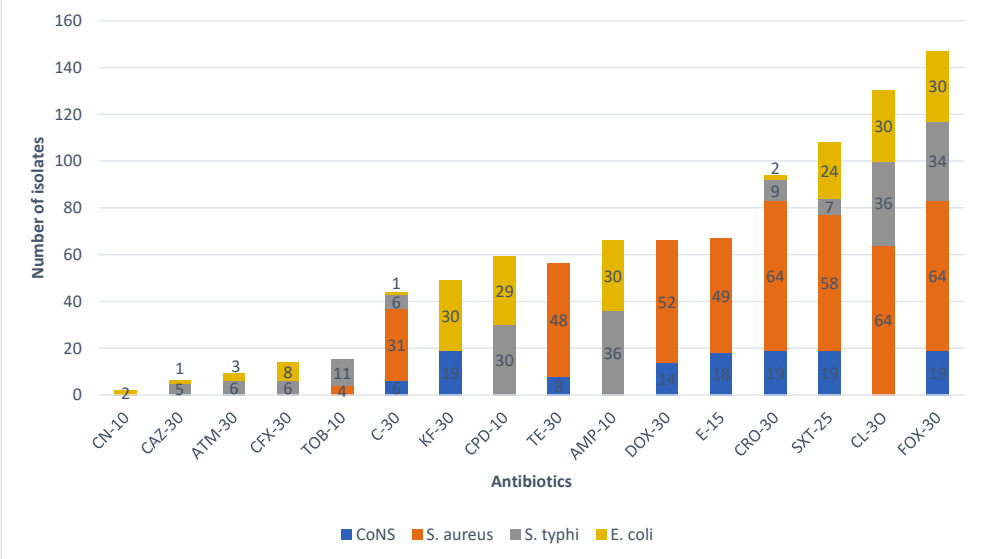

Fig. (2). Antibiograms of bacterial isolates from water samples used on poultry farms.

Table 1. Antibiotic resistant profiles of bacterial isolates from samples of different sources of water.

Antibiotic (µg)	Source of Resistant Isolates (Frequency)														
	Tap Water Isolates				Well Water Isolates				Borehole Isolates				Stream Water Isolates		
	EC (7)	ST (11)	SA (20)	CoNS (6)	EC (12)	ST (13)	SA 22)	CoNS (8)	EC (12)	ST (11)	SA (21)	CoNS (5)		ST (1)	SA (1)
SXT-25	5	3	19	6	10	1	18	8	9	2	20	5		1	1
TOB-10	0	7	1	0	0	1	2	0	0	2	1	0		1	0
CAZ-30	0	4	0	0	1	0	0	0	0	1	0	0		0	0
AMP-10	7	11	0	0	12	12	0	0	11	11	0	0		1	0
NOR-10	0	0	0	0	0	0	0	0	0	0	0	0		0	0
KF-30	7	0	20	6	12	0	22	8	11	0	21	5		0	1
FOX-30	7	11	20	6	12	11	22	8	11	10	21	5		1	1
C-30	0	3	8	2	0	2	10	2	1	0	12	2		1	1
CRO-30	0	5	20	6	0	1	22	8	2	2	21	5		1	1
CFX-30	0	3	0	0	4	1	0	0	4	1	0	0		1	0
CN-10	0	0	0	0	1	0	0	0	1	0	0	0		0	0
CPD-10	7	10	0	0	10	9	0	0	12	10	0	0		1	0
ATM-30	0	3	0	0	2	1	0	0	1	1	0	0		1	0
CIP-5	0	0	0	0	0	0	0	0	0	0	0	0		0	0
CL-30	7	11	0	0	12	12	0	0	11	11	0	0		1	0
E-15	0	0	19	5	0	0	16	8	0	0	13	5		0	1
DOX-30	0	0	18	5	0	0	16	5	0	0	18	4		0	1
TE-30	0	0	15	2	0	0	16	4	0	0	16	2		0	1

SXT-25: Sulphamethoxazole+Trimethoprim-25 µg; TOB-10: Tobramycin-10 µg; CAZ-30: Ceftazidime-30 µg; AMP-10: Ampicillin-10 µg NOR-10:Norfloxacin-10 µg, KF-30: Cephalothin-30 µg; g, FOX-30:Cefoxitin-30 µg;, C-30: Chloramphenicol-30 µg;, CRO-30:Ceftriaxone-30 µg;, CFX-30:Cefotaxime-30 µg;, CN-10: Gentamycin-10 µg, CPD-10:Cefpodoxime-10 µg;, ATM-30:Aztreonam-30 µg;, CL-30:Cephalexin-30 µg; CIP-5:Ciprofloxacin-5 µg;, DOX-30:Doxycycline-30 µg;, E-15:Erythromycin-15 µg;, TE-30:Tetracycline-30 µg;, EC*: Escherichia coli,* SA*: Staphylococcus aureus,* ST*:Salmonella typhi,* CoNS*:*Coagulase-negative *Staphylococci*.

Thirty-six (100%) *S. typhi* isolates were resistant to cephalexin and ampicillin, whereas thirty-four (94.4%) and 30 (83.3%) isolates were resistant to cefoxitin and cefpodoxime, respectively. The numbers of *S. typhi* isolates resistant to tobramycin, ceftriaxone, sulphamethoxazole/trimethoprim were 11 (30.6%), 9 (25%) and 7 (19.4%), respectively. Six (16.7%) *S. typhi* isolates were resistant to aztreonam, cefotaxime and chloramphenicol while 5 (13.9%) of the isolates were resistant to ceftazidime (Fig. **2**).

All the sixty-four (100%) *S. aureus* isolates were resistant to ceftriaxone, cefoxitin and cephalexin. Fifty-eight (90.6%) and 52 (81.3%) *S. aureus* isolates were resistant to sulphamethoxazole/trimethoprim and doxycycline, respectively, whereas 49 (76.6%) *S. aureus* isolates were resistant to erythromycin. Resistance to tetracycline, chloramphenicol and tobramycin was seen in 48 (75%), 31 (48.4%) and 4 (6.3%) *S. aureus* isolates, respectively. All sixty-four (100%) *S. aureus* isolates were susceptible to ciprofloxacin and norfloxacin (Fig. **2**).

All the nineteen (100%) Coagulase-Negative *Staphylococci* (CoNS) were resistant to sulphamethoxazole /trimethoprim, ceftriaxone, cephalothin and cefoxitin, whereas 18 (94.7%) isolates were resistant to erythromycin. *S. aureus* resistance to doxycycline, tetracycline and chloramphenicol was observed in 14 (73.7%), 8 (42.1%) and 6 (31.6%) CoNS isolates, res-pectively (Fig. **2**). The distribution of these resistant isolates per sources of water is represented in Table **1**.

Table 2. Antibiotic resistance profiles of *S. typhi* isolates.

Number of Antibiotics (Frequency)	Sources of Antibiotic Resistant Isolates										
	Source of Water (Number of Isolates)									Total Isolates (n=36)	
	Antibiotics	Tap Water (n=12)		Well Water (n=12)		Stream Water (n=1)		Borehole (n=11)			
		N	%	N	%	N	%	N	%	N_T	%
3 (4)	CL/FOX/AMP	0	0	1	8.3	0	0	1	9.1	2	5.6
	CL/AMP/CPD	0	0	0	0	0	0	1	9.1	1	2.8
	CL/TOB/AMP	0	0	1	8.3	0	0	0	0	1	2.8
4 (18)	CL/FOX/AMP/CPD	5	41.7	7	58.3	0	0	5	45.5	17	47.2
	CL/FOX/C/AMP	0	0	1	8.3	0	0	0	0	1	2.8

(Table 2) contd.....

Number of Antibiotics (Frequency)	Sources of Antibiotic Resistant Isolates										
	Source of Water (Number of Isolates)									Total Isolates (n=36)	
	Antibiotics	Tap Water (n=12)		Well Water (n=12)		Stream Water (n=1)		Borehole (n=11)			
		N	%	N	%	N	%	N	%	N_T	%
5 (5)	CL/FOX/TOB/AMP/CRO	1	8.3	0	0	0	0	0	0	1	2.8
	CL/FOX/AMP/CRO/CPD	0	0	1	8.3	0	0	0	0	1	2.8
	CL/FOX/TOB/AMP/CPD	0	0	0	0	0	0	1	9.1	1	2.8
	CL/FOX/AMP/SXT/CPD	0	0	0	0	0	0	1	9.1	1	2.8
	CL/FOX/TOB/AMP/ATM	1	8.3	0	0	0	0	0	0	1	2.8
7 (2)	CAZ/CL/FOX/AMP/SXT/CRO/CPD	0	0	0	0	0	0	1	9.1	1	2.8
	CL/FOX/TOB/C/AMP/CRO/CPD	1	8.3	0	0	0	0	0	0	1	2.8
8 (2)	CL/FOX/TOB/AMP/CRO/ATM/CPD/CFX	0	0	0	0	0	0	1	9.1	1	2.8
	CAZ/CL/FOX/TOB/C/AMP/CRO/CPD	1	8.3	0	0	0	0	0	0	1	2.8
9 (3)	CAZ/CL/FOX/TOB/AMP/CRO/ATM/CPD/CFX	2	16.7	0	0	0	0	0	0	2	5.6
	CL/FOX/C/AMP/SXT/CRO/ATM/CPD/CFX	0	0	1	8.3	0	0	0	0	1	2.8
10 (2)	CAZ/CL/FOX/TOB/C/AMP/SXT/CRO/CPD/CFX	1	8.3	0	0	0	0	0	0	1	2.8
	CL/FOX/TOB/C/AMP/SXT/CRO/ATM/CPD/CFX	0	0	0	0	1	100	0	0	1	2.8

SXT-25: Sulphamethoxazole+Trimethoprim-25 µg; TOB-10: Tobramycin-10 µg; CAZ-30: Ceftazidime-30 µg; AMP-10: Ampicillin-10 µg; FOX-30: Cefoxitin-30 µg; C-30 Chloramphenicol-30 µg; CRO-30: Ceftriaxone-30 µg; CFX-30: Cefotaxime-30 µg; CPD-30: Cefpodoxime-30 µg; ATM-30: Aztreonam-30 µg; CL-30: Cephalexin-30 µg; n: Total number of *S. typhi* isolates per source of water; N: Number of resistant isolates from water source; N_T: Total number of isolates per category of resistance.

3.3. Multi-drug Resistant Isolates from Various Sources of Water

Multi-drug resistant isolates were defined as isolates resistant to more than 3 antibiotics. Thirty- two (88.9%) *S. typhi* isolates were identified to be multi-drug resistant strains. Eighteen (50%) *S. typhi* isolates were resistant to 4 antibiotics, while 5 (13.9%) isolates were resistant to 5 antibiotics. Two (5.6%) isolates each were resistant to 7, 8 and 10 antibiotics while three (8.3%) isolates were resistant to 9 antibiotics (Table **2**). Sixty-one (95.3%) *S. aureus* isolates were identified to be multi-drug resistant strains. Two (3.1%), 3 (4.7%) and 13 (20.3%) isolates were resistant to 4, 5 and 6 antibiotics, respectively. Twenty-one isolates each were resistant to 7 and 8 antibiotics with 1 (1.6%) isolate resistant to 9 antibiotics (Table **3**). Nineteen (100%) CoNS isolates were multi-drug resistant, of which twelve (63.2%) CoNS isolates were resistant to 6 antibiotics. Six (31.6%) and 1 (5.3) CoNS isolates were resistant to 7 and 8 antibiotics, respectively (Table **4**). Thirty-one (100%) *E. coli* isolates were multi-drug resistant. One (3.2%) isolate was resistant to 4 antibiotics. Five (16.1%), 17 (54.8%) and 6 (19.4%) isolates were resistant to 5, 6 and 7 antibiotics, respectively. One (3.2%) isolate each was resistant to 8 and 9 different antibiotics, respectively (Table **5**).

Table 3. Antibiotic resistance profiles of *S. aureus* isolates .

Number of Antibiotics (Frequency)	Sources of Antibiotic Resistant Isolates										
	Source of Water (Number of Isolates)									Total Isolates (n=64)	
	Antibiotics	Tap (n=20)		Well (n=22)		Stream (n=1)		Borehole (n=21)			
		N	%	N	%	N	%	N	%	N_T	%
3 (3)	CRO/FOX/KF	0	0	2	9.1	0	0	1	4.8	3	4.7
4 (2)	SXT/CRO/FOX/KF	0	0	0	0	0	0	1	4.8	1	1.6
	E/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
5 (3)	TE/E/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
	SXT/E/CRO/FOX/KF	0	0	0	0	0	0	1	4.8	1	1.6
	SXT/DOX/CRO/FOX/KF	1	3	0	0	0	0	0	0	1	1.6
6 (13)	TE/E/DOX/CRO/FOX/KF	1	5	0	0	0	0	0	0	1	1.6
	SXT/C/E/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
	SXT/TE/E/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
	SXT/C/DOX/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
	SXT/E/DOX/CRO/FOX/KF	3	15	1	4.5	0	0	0	0	4	6.3
	SXT/TE/DOX/CRO/FOX/KF	0	0	2	9.1	0	0	3	14.3	5	7.8

(Table 3) contd.....

Number of Antibiotics (Frequency)	Sources of Antibiotic Resistant Isolates										
	Source of Water (Number of Isolates)									Total Isolates (n=64)	
	Antibiotics	Tap (n=20)		Well (n=22)		Stream (n=1)		Borehole (n=21)			
		N	%	N	%	N	%	N	%	N_T	%
7 (21)	SXT/TE/E/DOX/CRO/FOX/KF	6	30	4	18.2	0	0	3	14.3	13	20.3
	SXT/TE/C/DOX/CRO/FOX/KF	0	0	1	4.5	0	0	3	14.3	4	6.3
	SXT/C/E/DOX/CRO/FOX/KF	1	5	0	0	0	0	1	4.8	2	3.2
	SXT/TE/TOB/E/CRO/FOX/KF	1	5	0	0	0	0	0	0	1	1.6
	SXT/TE/C/E/CRO/FOX/KF	1	5	0	0	0	0	0	0	1	1.6
8 (21)	SXT/TE/C/E/DOX/CRO/FOX/KF	6	30	5	22.7	1	100	7	33.3	19	29.7
	SXT/TOB/C/E/DOX/CRO/FOX/KF	0	0	0	0	0	0	1	4.8	1	1.6
	SXT/TE/TOB/C/E/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6
9 (1)	SXT/TE/TOB/C/E/DOX/CRO/FOX/KF	0	0	1	4.5	0	0	0	0	1	1.6

SXT-25: Sulphamethoxazole/Trimethoprim-25 µg; DOX-30: Doxycycline-30 µg; E-15: Erythromycin-15 µg; TOB-10: Tobramycin-10 µg; TET-30: Tetracycline-30 µg; FOX-30: Cefoxitin-30 µg; C-30: Chloramphenicol-30 µg; CRO-30: Ceftriaxone-30 µg; KF-30: Cephalothin-30 µg; n: Total number of *S. aureus* isolates per source of water; N: Number of resistant isolates from water source; N_T: Total number of isolates per category of resistance.

Table 4. Antibiotic resistance profiles of CoNSisolates.

Number of Antibiotics (Frequency)	Sources of Antibiotic Resistant Isolates							
	Source of Water (Number of Isolates)						Total Isolates n=19	
	Antibiotics	Tap (n=6)		Well (n=8)		Borehole (n=5)		
		No. of isolates	% per source	No. of isolates	% per source	No. of isolates	% per source	Total	% per total
6 (12)	SXT/DOX/CRO/E/KF/FOX	2	33.3	3	37.5	3	60	8	42.1
	SXT/CRO/E/KF/TE/FOX	0	0	2	25	0	0	2	10.6
	SXT/CRO/E/KF/FOX/C	1	16.7	0	0	0	0	1	5.3
	SXT/DOX/CRO/KF/FOX/C	1	16.7	0	0	0	0	1	5.3
7 (6)	SXT/DOX/CRO/E/KF/TE/FOX	2	33.3	1	12.5	0	0	3	15.9
	SXT/CRO/E/KF/TE/FOX/C	0	0	1	12.5	1	20	2	10.6
	SXT/DOX/CRO/E/KF/FOX/C	0	0	1	12.5	0	0	1	53
8 (1)	SXT/DOX/CRO/E/KF/TE/FOX/C	0	0	0	0	1	20	1	5.3

SXT-25: Sulphamethoxazole/Trimethoprim-25 µg; DOX-30: Doxycycline-30 µg; E-15: Erythromycin-15 µg; TOB-10: Tobramycin-10 µg; TE-30: Tetracycline-30 µg; FOX-30: Cefoxitin-30 µg; C-30: Chloramphenicol-30 µg; CRO-30: Ceftriaxone-30 µg; KF-30: Cephalothin-30 µg; n: Total number of CoNS isolates per source of water; N: Number of resistant isolates from water source; N_T: Total number of isolates per category of resistance.

3.4. Detection of ESBL Producing *E. coli* and *S. typhi* Isolates

None of the bacterial isolates was found to be ESBL-producing organism.

4. DISCUSSION

The ubiquitous nature of microbial agents accounts for their easy contamination of substances that are used by both humans and non-humans. Depending on their rate of contamination, various infections may arise [17]. Water is an abundant resource used for farming activities such as human consumption, domestic purposes, including its use in poultry farming, *etc.* Physical, chemical and microbial parameters of the water may affect the various uses of water for human and non-human activities. Various degrees of contamination with total coliforms, faecal coliforms and enterococci have been found in hand-filled-hand-knotted water sold on the streets of Kumasi [18]. A study by Osei *et al.* [11] identified various levels of microbial contaminants in water used for poultry production in poultry farms in the Ashanti region of Ghana. Osei *et al.* [11] also reported that 91% of the water used on poultry farms in Ghana had various levels of microbial contamination with one or more microorganisms including *E. coli, S. typhi, S. aureus* and coagulase-negative *Staphylococci*. These microbial contaminants were found in 91.42% borehole water samples, 90.91% of well water, 90.32% of tap water and 100% of stream water samples collected. Thirty-one *E. coli,* 36 *S. typhi,* 64 *S. aureus* and 19 coagulase-negative Staphylococcal isolates were identified, respectively. The presence of such pathogenic organisms in water, coupled with possible antibiotic resistance, is of public health importance [19].

Table 5. Antibiotic sensitivity profile of *E. coli* isolates.

Number of Antibiotics (Frequency)	Source of Antibiotic Resistant Isolates								
	Source of Water (Number of isolates)							Total Isolates (n=31)	
		Tap Water (n=7)		Well Water (n=12)		Borehole Water (n=12)			
	Antibiotics	N	%	N	%	N	%	N_T	%
4 (1)	SXT/CRO/CPD/ATM	0	0	0	0	1	8.3	1	3.2
5 (5)	AMP/KF/FOX/CPD/CL	2	28.6	1	8.3	1	8.3	4	12.9
	SXT/AMP/KF/FOX/CL	0	0	0	0	1	8.3	1	3.2
6 (17)	SXT/AMP/KF/FOX/CPD/CL	5	71.4	6	50	5	41.7	16	51.6
	AMP/KF/FOX/CN/ATM/CL	0	0	1	8.3	0	0	1	3.2
7 (6)	SXT/AMP/KF/FOX/CFX/ CPD/CL	0	0	3	25	3	25	6	19.4
8 (1)	AMP/KF/FOX/C/CFX /CN/CPD/CL	0	0	0	0	1	8.3	1	3.2
9 (1)	CAZ/AMP/KF/FOX/CRO/CFX /CPD/ATM/CL	0	0	1	8.3	0	0	1	3.2

SXT-25: Sulphamethoxazole+Trimethoprim-25 µg; CAZ-30: Ceftazidime-30 µg; AMP-10: Ampicillin-10 µg; KF-30: Cephalothin-30 µg; FOX-30: Cefoxitin-30 µg; C-30: Chloramphenicol-30 µg; CRO-30: Ceftriaxone-30 µg; CFX-30: Cefotaxime-30 µg; CN-10: Gentamycin-10 µg; CPD-10: Cefpodoxime-10 µg; ATM-30: Aztreonam-30 µg; CL-30: Cephalexin-30 µg; n: Total number of *E. coli* isolates per source of water; N: Number of resistant isolates from water source; N_T: Total number of isolates per category of resistance.

These findings confirm the report by Arhin-Sam [20], which indicated that various enterobacterial species were identified in samples of groundwater used as drinking water within the Kumasi Metropolis of Ghana.

In Ghana, the quality of groundwater and surface water resources keeps deteriorating largely due to increasing levels of pollution from "galamsey" sites (illegal mining), untreated sewage and industrial waste, leachate from fertilisers and pesticides used in agriculture, and the use of chemicals in fishing. It is undeniable that Ghana's freshwater resources are seriously under threat as water resources are running dry and overwhelmingly becoming scarce day by day. This has consequently forced some managers of water treatment plants in Ghana to shut down due to pollution, which makes the cost of water treatment very expensive [21]. This has unfortunately necessitated the use of untreated water on animal farms to reduce the cost of production while increasing the risk of microbial infection among farm animals and workers, cross infection from animals to workers and increased microbial contamination of animal products [11].

All *S. typhi* isolates were resistant to cephalexin and ampicillin followed by cefoxitin (94.4%) and cefpodoxime (83.3%). There were low levels of resistance to ceftazidime (13.9%), aztreonam (19.4%), chloramphenicol (19.4%) and cefotaxime (19.4%), but none was resistant to norfloxacin. These findings support a report by Thung *et al.* [22] where *S. typhi* isolates obtained from raw chicken meat in retail markets in Malaysia were reported to be highly resistant to ampicillin, vancomycin and penicillin but moderately sensitive to antibiotics such as ceftazidime and tetracycline with the majority of these isolates being multi-drug resistant isolates. Almost 90% of *S. typhi* isolates were multi-drug resistant, which is in contrast to the findings of M'ikanatha [23] where 31% of *S. typhi* isolates obtained from retail poultry in the USA were multi-drug resistant. This high (88.9%) percentage of multi-drug resistant bacterial isolates can be attributed to the constant increase in the misuse of antibiotics in most developing countries, especially in animal husbandry [24].

Over 75% of *S. aureus* isolates were resistant to the reference antibiotics used. This high percentage is consistent with the report by Olufemi *et al*. [25], where over 80% *S. aureus* isolated from recreational waters and beach sand in eastern South Africa were resistant to the standard antibiotics used in the study. The occurrence of multi-drug resistant isolates (95%) in this current study is higher than the 52% reported by Waters *et al*. [26] in meat and poultry in the United States. This may be attributed to a constant increase in the misuse of antibiotics in most developing countries, especially in animal husbandry [24].

All the coagulase-negative Staphylococcal isolates were resistant to sulphamethoxazole-trimethoprim, ceftriaxone, cephalexin and cefoxitin but susceptible to norfloxacin and tobramycin. Moreover, all isolates were multi-drug resistant, with 63.2% being resistant to six antibiotics. Boamah [4] reported that Coagulase-Negative Staphylococcal (CoNS) isolates from poultry litter were resistant to similar antibiotics in Ghana. The percentage (100%) of multi-drug resistant CoNS isolates was higher compared to 65% reported by Boamah [4] and this can be attributed to the acquisition of resistant genes from strains of different organisms over the period and reported increases in the misuse and abuse of antibiotics in most developing countries including Ghana [24].

All isolates of *E. coli* were susceptible to tobramycin, norfloxacin and ciprofloxacin. Almost 97% of the isolates were resistant to ampicillin, cephalexin, cephalothin, cefoxitin and cefpodoxime but exhibited a low level of resistance towards ceftriaxone (6.45%), chloramphenicol (3.22%) and ceftazidime (3.22%). All *E. coli* isolates were multi-drug resistant. Fifty-five percent of isolates were resistant to 6 of the reference antibiotics. The findings of this study confirm the pattern of antibiotic resistance in *E. coli* isolates obtained from water similar to a study by Sayah *et al*. [27] which reported that *E. coli* isolates from surface water, domestic and wild animals and faecal samples, were found to be resistant to antibacterial

agents such as ampicillin, chloramphenicol, tetracycline, sulphamethoxazole/trimethoprim, cephalothin, neomycin and streptomycin. These findings are consistent with a report by Wose-Kinge *et al*. [28] on the antibiotic resistance profiles of *E. coli* isolated from different water sources in the Mmabatho locality in South Africa, where these isolates were identified to be resistant to antibiotics such as erythromycin, tetracycline, ampicillin and chloramphenicol. The occurrence of multi-drug resistant *E. coli* is similar to a report by Ogunleye *et al*. [29] where all isolates of *E. coli* from poultry in Abeokuta, Southwestern Nigeria were also found to be multi-drug resistant.

None of the *E. coli* and *S. typhi* isolates were found to be extended-spectrum beta lactamase producing bacteria, which confirms the report by Boamah [4] where none of the enterobacterial species from poultry litter from poultry farms in Ghana was an ESBL producing bacteria. This implies that the resistance of the isolates to beta-lactam antibiotics cannot be attributed to the production of extended spectrum beta-lactamases. There is a need to determine the possible mechanism(s) of resistance for these multi-drug resistant bacterial strains.

CONCLUSION

Antibiotic-resistant and multi-drug resistant *E. coli, S. typhi, S. aureus* and coagulase-negative *Staphylococci* isolates were identified and none of the isolates was found to produce extended spectrum beta-lactamases. All necessary strict measures should be taken by relevant stakeholders involved in the use, regulation and monitoring of antibiotics in animal husbandry and microbial quality assessment of water used in the poultry industry should be done to prevent the transmission of infections to both animals and humans.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data, including the supplementary data supporting the findings of the article, is available from the Microbiology Section of Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana at https://pharmacy.knust.edu.gh/departments/pharmaceutics.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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