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Prevalence and antibiotic susceptibility of *Salmonella* spp. from water sources in Tamale, Ghana

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Abstract

Aim: This study investigated the prevalence and antibiotic resistance of *Salmonella* species isolated from drinking water sources in Tamale Metropolis.

Materials and Methods: Isolation of *Salmonella* species from 275 different drinking water samples (25 each from dam, well, rain, and bottle, 35 from tap, 40 from water trough, and 100 from sachet) was done using a slightly modified method of the Bacteriological Analytical Manual of the Food and Drugs Administration, USA. 34 *Salmonella* species isolated from the water samples were examined for their susceptibility to nine different antibiotics using the disc diffusion method. The study was carried out from July 2014 to January 2015.

Results: The overall prevalence of *Salmonella* species was 4.36% (12/275). Dam 16.00% (4/25) and well 16.00% (4/25) water samples were the most contaminated source, followed by rain water (stored) 12.00% (3/25) and tap water samples 2.86% (1/35). There were no significant differences among water samples which were positive for *Salmonella* species ($p>0.05$); however, dam and well samples that were positive for *Salmonella* species differ significantly ($p<0.05$) from bottle water, sachet water, and water trough samples, which were negative for *Salmonella* species. The 34 *Salmonella* isolates were highly resistant to erythromycin (E) (100%) and vancomycin (VA) (94.12%). Few isolates exhibited intermediate resistances to ceftriaxone (CRO) (17.65%), gentamicin (CN) (17.65%), tetracycline (14.71%), chloramphenicol (C) (5.88%), ciprofloxacin (CIP) (2.94%), and amoxicillin (AMC) (2.94%). *Salmonella* isolates also exhibited six different antibiotic resistant patterns (VA-E, VA-E-AMC, VA-E-CRO, VA-E-C, VA-E-CRO-AMC, and VA-E-AMC-CN). The resistant pattern VA-E (with multiple antibiotic resistance index of 0.22) was the commonest.

Conclusion: This study indicated that some drinking water sources for humans and animals in Tamale Metropolis are contaminated with *Salmonella* species which exhibited varying resistance to various antibiotics. Therefore, consumers of water at the Tamale Metropolis are at risk of *Salmonella* infection from drinking water from positive water sources in the Tamale Metropolis.

Keywords: antibiotics, drinking water, public health, *Salmonella* species.

Introduction

Water is very essential for the existence of humans and other forms of life on earth [1]. It is involved in the normal functioning of cells that make up humans and all living organisms [2,3]. Water also helps to remove waste, lubricates joints, serve as a major component of blood, and a necessary medium for many chemical reactions that help in the formation of meat, eggs, and other animal and plant foods [4]. Potable water is expected to be odorless, tasteless, free from toxic substances, microbial and chemical contaminations [2,5]. Water is also known to be the dwelling place for most bacteria and other microorganisms which cause a variety of waterborne infections such as cholera, typhoid, bilharzia, and malaria [6,7].

Salmonella species are Gram-negative facultative anaerobe bacteria and have been isolated from humans, animals, and the environment [8-14]. *Salmonellae* are among the major pathogenic bacteria in humans as well as in animals [15,16]. They are the leading cause of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide [15]. In Canada, 8.5% (342) of river water samples were contaminated with *Salmonella* species which were influenced by rainfall runoff and drainage from agricultural land [17]. In Czech, 16% (87) of pond water samples were contaminated with *Salmonella* species [18]. In South Africa, the prevalence rate of *Salmonella* species in different river samples ranged from 33-90% [19]. A study by Akinyemi *et al*. [20] in Nigeria indicated that *Salmonella* was present in water samples. Hitherto published work on the isolation and antibiotic resistance of *Salmonella* species in Ghana appears to be unavailable. Antibiotics continue to play a very important role in decreasing diseases, illness and/or death associated with bacterial infections [21]. Human activities have been largely linked to the emergence of multidrug resistance isolates [16,22].

Salmonella species isolated from water samples were resistant to 2 or more antibiotics [21]. This study was conducted to find out whether drinking water sources in the Tamale, Metropolis of Ghana are contaminated with *Salmonella* species. The study also investigated the antibiotic resistance of the *Salmonella* species isolated from the water samples.

Materials and Methods

Ethical approval

All samples were collected as per the standard sample collection procedure without harming humans or animals.

Location of study

The study was conducted in Tamale Metropolis. Tamale is the capital town of the Northern Region of Ghana. It is located within the Guinea Savannah belt. Tamale is the third most populous settlement in Ghana, in terms of population, with a population of 537,986 people [23]. Tamale Metropolis can be located on longitude 09°24′27″ North and latitude 00°51′12″ West [23]. The Tamale Metropolis occupies approximately 750 km^2, which is 13% of the total area of the Northern Region [23].

Sample collection

A total of 275 water samples were sampled. These water samples were collected aseptically from dam (n=25), well (n=25), rain (n=25), tap (n = 35), bottle (n=25), sachet (n=100), and water trough (n=40). The water samples were stored at 4°C and transported to the Spanish Laboratory of University for Development Studies, Nyankpala, Ghana, where analysis was carried out immediately upon arrival for the presence of *Salmonella* species.

Bacteriological analysis

Analyses for *Salmonella* species were done using a modified method according to the Bacteriological Analytical Manual of the Food and Drug Administration [8]. 500 ml of the various water samples were obtained and thoroughly mixed. 1 ml of water was taken from the 500 ml, transferred into 10 ml buffered peptone water, and incubated at 37°C for 24±2 h. Afterward, 0.1 ml and 1 ml of pre-enriched aliquots were transferred into 10 ml Rappaport and Vassiliadis broth and 10 ml selenite cystine broth, respectively. Enrichment samples in Rappaport and Vassiliadis broth were incubated at 42°C for 24 h while that of the selenite cystine broth were incubated at 37°C for 24 h. Enriched aliquots (ca. 10 µl) were then streaked onto xylose lysine deoxycholate and brilliant green bile agar and incubated at 37°C for 24-48 h. Presumptive *Salmonella* species were purified on nutrient agar and were identified and confirmed using Gram-staining, biochemical tests, and *Salmonella* latex agglutination test. All incubations were done under aerobic condition, and all media used were purchased from Oxoid, Oxoid Limited, Basingstoke, UK.

Antimicrobial susceptibility of *Salmonella* species

The disk diffusion method of Bauer *et al*. [24] was used to determine the antibiotic resistance of 34 *Salmonella* species against the following antibiotics: Amoxicillin/clavulanic acid (AMC) 30 µg, chloramphenicol (C) 30 µg, gentamicin (CN) 10 µg, ceftriaxone (CRO) 30 µg, ciprofloxacin (CIP) 5 µg, erythromycin (E) 15 µg, sulfamethoxazole/trimethoprim (SXT) 22 µg, tetracycline (TE) 30 µg, and vancomycin (VA) 30 µg. The disks were purchased from Oxoid Limited, Basingstoke, UK. Pure cultures of *Salmonella* species were grown overnight in tryptic soy broth (TSB) (Oxoid Limited, Basingstoke, UK) at 37°C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. About 100 µl of the culture was then swabbed onto Mueller-Hinton agar (Oxoid Limited, Basingstoke, UK) using a sterile cotton swab. Three antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16-18 h, and the results were interpreted as sensitive, intermediate, or resistance according to the Clinical and Laboratory Standards Institute Guidelines [25]. The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman [26] using the formula: a/b, where "a" represents the number of antibiotics to which a particular isolate was resistant and "b" the total number of antibiotics tested.

Statistical analysis

The data obtained were analyzed using the generalized linear model of Statistical Package for the Social Sciences (SPSS) Version 17.

Results

The results for the occurrence of *Salmonella* species in water samples examined in the Tamale Metropolis are presented in Table-1. The overall prevalence of *Salmonella species* was 6.36% (12/275). Dam (16.00%) and well (16.00%) water samples were the most contaminated source, followed by rain water (12.00%) and tap water samples (2.86%). Bottle, sachet, and water trough samples recorded 0.00% (0/25), (0/100), and (0/40), respectively. There were no significant differences among water samples which were positive for *Salmonella* species ($p>0.05$);

Table-1: Distribution of *Salmonella* species in the various drinking water sources tested.

Sources of drinking water	Number of samples tested	Number of samples positive	Percentage prevalence
Dam water	25	4	16.00
Well water	25	4	16.00
Rain water	25	3	12.00
Tap water	35	1	2.85
Bottle water	25	0	0.00
Sachet water	100	0	0.00
Water trough	40	0	0.00
Total	275	12	4.36

however, dam and well water samples that were positive for *Salmonella* species differ significantly ($p<0.05$) from bottle water, sachet water, and water trough samples.

The antibiotic susceptibility test of the *Salmonella* isolates is shown in Table-2. Resistant to E and VA was 100% and 94.12%, respectively. None of the *Salmonella* isolates was resistant to SXT, TE, and CIP, even though intermediate resistances occurred for TE (14.71%) and CIP (2.94%). Most of the isolates were highly susceptible to C (91.18%), CN (79.41%), AMC acid (73.53%), and CRO (70.59%).

The 34 *Salmonella* isolates also exhibited six different antibiotic resistant patterns. 23 of the isolates exhibited a resistant pattern of VA-E with MAR index of 0.22, five exhibited a pattern of VA-E-AMC with MAR index of 0.33, two isolates exhibited a pattern of VA-E-CRO with MAR index of 0.33, one exhibited a pattern of VA-E-C with a MAR index of 0.33, two exhibited a pattern of VA-E-CRO-AMC with MAR index of 0.44, and one exhibited a pattern of VA-E-AMC-CN with MAR index of 0.44 (Table-3).

Discussion

Of the 275 different drinking water samples examined, 12 were positive for *Salmonella* species. The occurrence of *Salmonella* species was highest in dam and well water samples, followed by rain and tap water samples. The presence of *Salmonella* species in dam, well, rain, and tap water samples is worrying since the majority of the people and animals in the Tamale Metropolis, and its environs drink from them. It was observed that dams which were positive for *Salmonella* species were closer to human settlement and under high utilization by humans and animals. Some of the wells which were contaminated with *Salmonella* species were shallow, without proper cover and the containers used for fetching water from them were dirty and left on the ground. In this study, we collected rain water from stored containers/tanks and directly from the sky and roof (aluminum sheet) during raining. Water samples collected directly from the roof and the sky were negative for *Salmonella* species. Rainwater samples that were contaminated with *Salmonella* species were collected from storage containers/tanks. Containers used for fetching water from these storage containers/tanks were also sometimes dirty, left on the ground and reuse without washing. The containers used for fetching water from wells and storage tanks can easily get contaminated by soil and/or feces of domestic animals which were observed roaming around the wells and storage tanks in search of water. Ghana Water Company supplies water (tap water) to the various households in Tamale and its environs. This company ensures that the water supplied to consumers is of good quality without any contamination. One tap water sample collected from a chop bar was contaminated with *Salmonella* species. This contamination may be as a result of unhygienic handling of tap and personal hygiene of consumers. Indiscriminate defecation by humans and animals in and around water bodies can cause contamination of drinking water sources such as dams and wells. Dirts, chemicals, and/or feces created by humans and/or animal activities around water bodies can be washed back into these water bodies (dams and wells), especially by rains. Variation in the level of microorganisms in water bodies can be attributed to indiscriminate human and animal feces around such water bodies [27]. *Salmonella* species were not isolated from bottle water (four different brands), sachet water (four different brands), and water trough (from ruminant and poultry farms). This indicates that the bottle and sachet water samples were treated and packaged under good sanitary conditions without fecal or environmental contamination. The World Health Organization [28] reported that good quality drinking water should be free from microbial contamination. The absence of *Salmonella* species in the water trough samples could be attributed to the fact that appropriate management practices are being carried out at the farms visited. Frequent change of water and cleaning of water troughs create a good hygienic and sanitary condition which will prevent bacteria contamination and growth.

Around 34 *Salmonella* species isolated from the drinking water sources in the Tamale Metropolis were examined for their susceptibility to nine different antibiotics. They were highly resistant to E (100.00%) and VA (94.12%). Resistance to AMC was 23.53%. Few isolates also exhibited intermediate resistances to CRO (17.65%), CN (17.65%), TE (14.71%), C (5.88%), CIP (2.94%), and AMC (2.94%). Intermediate resistance means those *Salmonella* species were not

Table-2: Antibiotic susceptibility of drinking water *Salmonella* isolates.

Antibiotics	% Resistant	% Intermediate	% Susceptibility
Chloramphenicol	2.94	5.88	91.18
Gentamicin	2.94	17.65	79.41
Vancomycin	94.12	0.00	5.88
Sulfamethoxazole/trimethoprim	0.00	0.00	100.00
Tetracycline	0.00	14.71	85.29
Erythromycin	100.00	0.00	0.00
Ciprofloxacin	0.00	2.94	97.06
Ceftriaxone	11.76	17.65	70.59
Amoxicillin/clavulanic acid	23.53	2.94	73.53

Table-3: Antibiotic resistance pattern of the *Salmonella* species.

Code	Sources	Antibiotic resistance profile	MAR index
D1	Dam water	VA-E	0.22
D2	Dam water	VA-E	0.22
D3	Dam water	VA-E	0.22
D4	Dam water	VA-E	0.22
D5	Dam water	VA-E	0.22
D6	Dam water	VA-E	0.22
S1	Storage rain water	VA-E	0.22
S2	Storage rain water	VA-E	0.22
T1	Tap	VA-E	0.22
T2	Tap	VA-E	0.22
T3	Tap	VA-E	0.22
T4	Tap	VA-E	0.22
T5	Tap	VA-E	0.22
T6	Tap	VA-E	0.22
D7	Dam water	VA-E	0.22
D8	Dam water	VA-E	0.22
D9	Dam water	VA-E	0.22
D10	Dam water	VA-E	0.22
D11	Dam water	VA-E	0.22
S3	Storage rain water	VA-E	0.22
S4	Storage rain water	VA-E	0.22
S5	Storage rain water	VA-E	0.22
S6	Storage rain water	VA-E	0.22
W1	Well water	VA-E-AMC	0.33
W2	Well water	VA-E-AMC	0.33
W3	Well water	VA-E-AMC	0.33
W4	Well water	VA-E-AMC	0.33
S7	Storage rain water	VA-E-AMC	0.33
D12	Dam water	VA-E-CRO	0.33
D13	Dam water	VA-E-CRO	0.33
D14	Dam water	VA-E-C	0.33
W5	Well water	VA-E-CRO-AMC	0.44
S8	Storage rain water	VA-E-CRO-AMC	0.44
W6	Well water	VA-E-AMC-CN	0.44

C=Chloramphenicol, CN=Gentamicin, VA=Vancomycin, E=Erythromycin, CRO=Ceftriaxone, AMC=Amoxicillin/clavulanic acid, MAR=Multiple antibiotic resistance

clearly resistant or susceptible, and such isolates have the tendency to easily become resistant [29,30]. It has also been indicated that in clinical diagnoses patients with intermediate results can be given a higher dosage of antibiotics [29,30]. The 34 *Salmonella* isolates also exhibited six different antibiotic resistant patterns. The patterns were VA-E, VA-E-AMC, VA-E-CRO, VA-E-C, VA-E-CRO-AMC, and VA-E-AMC-CN. The resistant pattern VA-E was the most common and was exhibited by 23 *Salmonella* isolates. Resistant to as high as four different antibiotics (VA-E-CRO-AMC and VA-E-AMC-CN) was exhibited by 3 *Salmonella* isolates. *Salmonella species* are important pathogens of global concern, and the emergence of antibiotic resistant strains is largely due to indiscriminate use of antibiotics in animal feeds as growth promoters and as therapeutic agents for treating humans and animals [31].

Conclusion

This study indicated that some drinking water sources for humans and animals in Tamale Metropolis are contaminated with *Salmonella* species. In addition, they are resistant to some antibiotics at different rates. Therefore, drinking water sources in Tamale Metropolis are potential sources for the transmission of *Salmonella* infection.

Authors' Contributions

FA designed the experiment. CKNA and HA performed all the experiments. FA analyzed the data and wrote the draft manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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