

## Research Article

# Prevalence and Antimicrobial Susceptibility of Zoonotic *Salmonella* Species Isolated from Water Bodies in Bukombe District

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**Received:** May 10, 2018; **Accepted:** June 18, 2018;**Published:** June 25, 2018**Abstract**

A study on zoonotic *Salmonella* in water bodies was carried out between November 2016 and April 2017 in rural areas of Bukombe district, Tanzania. A total of 10 wards of Bukombe district were involved in the study. The main objective of the study was to estimate the burden of zoonotic *Salmonella* species (serovars Typhimurium and Enteritidis) as well as the antimicrobial resistance profile of isolates obtained from water bodies in Bukombe district. The specific objectives were to establish prevalence of zoonotic *Salmonella* spp from water, establish the antimicrobial resistance and to compare the prevalence of zoonotic *Salmonella* spp of multipurpose with single purpose water bodies in Bukombe district, Tanzania. A total of 240 water samples were examined for *Salmonella* contamination of which 50% were from multipurpose and the rest were from single purpose water bodies. *Salmonella* species were identified in 1.3% (3/240) of the 240 water samples. Multipurpose water bodies had a prevalence of 1.6% (2/120) while the single purpose water showed prevalence of 0.8% (1/120). The definitive confirmation by polymerase chain reaction (PCR) showed 1.3% isolates were positive for *Salmonella*. Based on biochemical test by lysine reaction 4.2% of the isolates were identified as *Salmonella* spp. The study observed that all isolated *Salmonella* spp. were resistant to penicillin (ampicillin) and cephalosporins (cephalexin), but were sensitive to florquinolones (ciprofloxacin) and aminoglycosides (gentamicin).

**Abbreviations and Symbols**

Spp: Species; PCR: Polymerase Chain Reaction; Var: Serovar; Subsp: Subspecies; n: Number of samples; rpm: Rotation per minute; DNA: Deoxyribose Nucleic Acid; Bp: Base pair; Min: Minute; sec: Second; V: Voltage; BSL: Biosafety Level; MDR: Multidrug Resistant; UK: United Kingdom; DWAF: Department of Water Affairs and Forestry in South Africa; HIV: Human Immunodeficiency Virus; WHO: World Health Organization; TBE: Tris-Borate-EDTA

**Introduction****Background information**

**Water bodies in Tanzania:** Most of the water bodies in Tanzania are poorly sanitized and protected which makes them potential sources of infections such as zoonotic *Salmonella*. The scarcity of water has also led people and animals to share the water body which increases chances of salmonellosis transmission in humans and animals [1]. As of 2012 only 40.6% of the households have access to improved drinking water sources such as piped water, tube wells and protected springs. The remaining percentage uses non-improved drinking water sources [2].

**Zoonotic salmonella:** *Salmonella* species are gram-negative, rod-shaped bacteria with broad host spectrum, including most animal species: mammals, birds and reptiles. Currently there are more than 2600 serotypes (serovars) of *Salmonella* classified into two species; *Salmonella enterica* and *Salmonella bongori*. *S. enterica* subsp.

*enterica* I is divided into serotypes, for example serotypes enteritidis, typhimurium, typhi and choleraesuis [3]. Serotypes *S. typhi* and *S. paratyphi A* are referred to as typhoidal *salmonella* the rest are called non-typhoidal or zoonotic *Salmonella* species.

**Epidemiology of zoonotic *Salmonella*:** Salmonellosis ranks high in causing bacterial enteric illness in humans and animals. Zoonotic *Salmonella* spreads by direct or indirect means from infected animals through contaminated feed and water supplies. Water is among the main transmission routes for diseases such as salmonellosis in humans and animals [4]. It is among the common causes of illness and death among the poor populations in developing countries. Individuals infected with *Salmonella* spp. shed the organism in their faeces, which may contaminate drinking water sources through rain runoff [5]. Infection can still occur even if concentration of *Salmonella* in water is low, because the organism escapes the natural host defense mechanisms due to rapid passage of water through the stomach into the intestines without stimulating digestion.

**Status of zoonotic *Salmonella*:** The prevalence of *salmonellas* is varies slightly from one country to another. For example in Nepal 42 out of 300 drinking water samples were positive, with a total of 54 isolates identified to genus level by PCR detection of the virulence genes *invA* and *spvC*. The predominant serotypes were *Salmonella typhimurium*, followed by *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella enteritidis* [6,7].

Salmonellosis is an important but neglected disease in sub-



**Figures 1:** Different water bodies in Bukombe district, single purpose uncovered water well in Namonge village (villagers fetching water and some laundering clothes) (A) and multipurpose water pond in Bukombe village, (cattle are seen drinking water) (B).

Saharan Africa, where food or fecal-oral associated transmissions are the primary causes of infections. The role of water-borne transmission is unclear, although significant prevalence has been demonstrated. For example, 6.5% of *Salmonella* spp was detected in dug wells in a rural area of Ghana [8]. A study by [9], showed the presence of *Salmonella* in water supplies to a slum and densely populated communities and a prevalence of 86 to 98.6% was reported in river Nile water in Egypt [10]. In Dar es salaam, Tanzania *Salmonella* spp. reported from different water bodies were; *Salmonella* ser. Paratyphi A (96.9%), and *Salmonella choleraesuis* serovar Choleraesuis (99.5%).

### Problem statement and justification

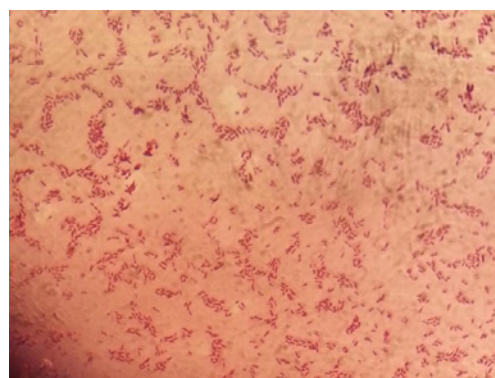
Tanzania faces a serious lack of potable water which forces people to share surface water sources with animals (livestock and wild) on a day-to-day basis [11]. Water bodies are therefore areas where zoonotic diseases, including salmonellosis, may arise. Bukombe district possess a number of livestock, which at some point share same water bodies with humans. At these water bodies infected animals may shed the bacteria through faeces. Also, humans contaminate the water while carrying out different activities including: Bathing, swimming, fishing and irrigation. These situations justify establishing the magnitude of water-borne zoonotic *Salmonella* species in both multi-purpose water bodies (with high human-animal interaction) and single-purpose water bodies (for humans use only) in communities such as Bukombe. So far limited studies have been conducted to address this concern in Bukombe district. This study aims at establishing the prevalence of zoonotic *Salmonella* spp. (serovars Typhimurium and Enteritidis) in the water bodies. Furthermore, the study determined the prevalence of antimicrobial resistance among *Salmonella* spp. isolated from the water bodies in Bukombe district.

### Objectives

**General objective:** To estimate the burden of zoonotic *Salmonella* species (serovars Typhimurium and Enteritidis) in water bodies and establish antimicrobial resistance profile of the bacteria isolated from water bodies in Bukombe district.

#### Specific objectives:

1. To estimate prevalence of zoonotic *Salmonella* species from single and multi-purpose water bodies in Bukombe district.
2. To establish the antimicrobial resistance profile of zoonotic



**Figure 2:** Gram stained smear showing gram negative rod shaped organisms at x100.

*Salmonella* species obtained from water bodies.

3. To compare the prevalence of zoonotic *Salmonella* spp of multipurpose versus single purpose water bodies in Bukombe district.

#### Research questions:

1. What is the prevalence of zoonotic *Salmonella* species in single and multi-purpose water bodies in Bukombe district?
2. What is the antimicrobial resistance profile of zoonotic *Salmonella* species obtained from the water bodies?
3. Is there any difference in the prevalence of zoonotic *Salmonella* species in multipurpose and single purpose water bodies?

## Literature Review

### Zoonotic *Salmonella*

*Salmonella* spp. comprise over 2600 serotypes and colonize range of animal hosts such as mammals, amphibians, reptiles, birds and insects [12]. The bacterial genus *Salmonella* causes a huge global burden of morbidity and mortality. With regard to human disease, *salmonellae* are divided into typhoidal serotypes (*Salmonella enteric* var Typhi) and *Salmonella enteric* var *Paratyphi* A) and thousands of non-typhoidal *Salmonella* serotypes [13]. Non-typhoidal *Salmonella* serotypes affect a wide range of hosts including human hence zoonotic, examples of these zoonotic *Salmonella* are *S. Typhimurium*

and *S. Enteritidis*.

### Epidemiology of zoonotic *Salmonella*

Water-borne diseases are a major public health concern worldwide, not only by the morbidity and mortality that they cause, but by the high cost associated with their prevention and treatment [14,15]. There has been increased number of epidemics in sub-Saharan Africa, with the tendency towards more typhoid-like with regard to patterns of transmission and virulence [16]. *Salmonella enterica* var. Typhimurium, ST313 and *Salmonella enteritidis* are far more important in sub-Saharan Africa. HIV infections in adults, malaria and malnutrition in children have been identified as important risk factors [12].

A distinct genotype of *Salmonella enterica* var. Typhimurium, ST313, has emerged as a new pathogenic clade in sub-Saharan Africa. This genotype might have adapted to cause invasive disease in humans [12]. According to [17], predominant serotypes have been shown to be *Salmonella enterica* serotype Typhimurium (54.9%) and *Salmonella enterica* serotype Enteritidis (64; 33.2%). It is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* species occur globally each year, causing 155,000 deaths [18].

According to the WHO, the mortality of water associated diseases exceeds five million people per year with 50% of these deaths being microbial intestinal infections. *Salmonella enterica* subsp. Enterica serovar Enteritidis is the most frequently isolated serovar from humans all over the world. Humans can carry the bacteria in the gut without showing signs of disease for considerable periods of time [19]. Water-borne *Salmonella* spp is becoming a big problem. A study by [20], detected a relatively low contamination frequency of *Salmonella* spp in ground water around burial sites of culled animals in South Korea. Some studies have revealed high numbers of water-borne pathogens, including 6% *Salmonella* spp in harvested rainwater, which is gaining acceptance among many countries such as Australia, Germany, and South Africa [21]. *Salmonella* species loads in borehole water in South Africa were higher than the WHO threshold, which suggests that water from these sources may pose serious health risks if consumed without treatment [22]. It is the aim of this study therefore to estimate the magnitude of zoonotic *Salmonella* spp in single and multipurpose water bodies in Bukombe district.

### Mode of infection and clinical signs

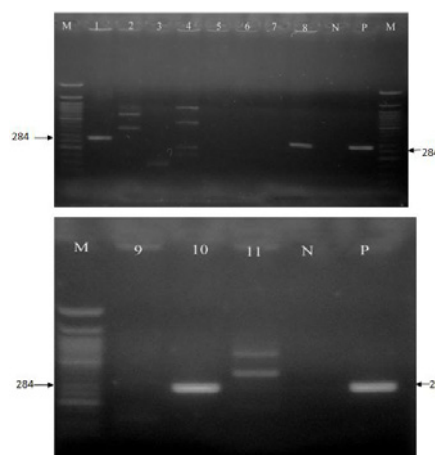
Most human *Salmonella* infections result in gastroenteritis, and are caused by *Salmonella* enteric serovar Typhimurium or *Salmonella* enteric serovar Enteritidis acquired from contaminated food and water. Apart from humans, other animals can also be infected with *Salmonella* Typhimurium. Some *Salmonella* spp are important pathogens that can be transmitted to people via food and water and can cause diseases characterized by mild to severe enteric and systemic illness [11]. *Salmonella* spp can arise from direct or indirect animal contact via an environment contaminated by clinically affected animals that exhibit a higher prevalence of shedding than apparently healthy animals, or transmission can occur through contaminated food and water to humans [23].

### Diagnostic methods of *Salmonella* species

Several diagnostic methods currently in use for *Salmonella* species; bacterial culture by using selective culture media such as



**Figure 3:** Colour change of lysine medium slants due to the presence of *Salmonella* spp. after incubation overnight at 37°C.



**Figure 4:** PCR products targeting *invA* gene at 284bp, on 1.5% of agarose gels for 1hr at 110V; ladder (M); positive for *Salmonella* spp. At 284bp (1, 8 and 10); negative control (N); positive control (P).

Xylose Lysine Deoxycholate (XLD) and *Salmonella* Shigella Agar (SSA). However, this method is time consuming and laborious [24].

PCR technology is widely used in detecting the presence of specific *Salmonella* species by amplification of a specific fragment of nucleic acid, genes such as *invA*, *fliC* are frequently targeted [25].

Serological methods are also used for the detection of *Salmonella* serotypes, one of them is ELISA, which may be indirect or double antibody-blocking assays using a range of antigens such as lipopolysaccharide, flagella and SEF14 fimbrial antigen [26].

## Materials and Methods

### Study area description

This study was conducted in Bukombe district which is located in the southern part of Geita region at the coordinates 03028'S 031054'E. The district has a total area of 8,055.59 km<sup>2</sup>. According to the 2012 census, the district has 75,668 cattle, 70,810 goats and 17,000 sheep and a human population of 224,542 individuals. The district is bordered to the East by Mbogwe District (Shinyanga), to the North by Chato District Geita, to the North-west by Biharamulo (Kagera) to the West by Kibondo District (Kigoma) and to the South by Urambo District (Tabora). The district eastern part constitutes Bugelenga, Bukombe, Butinzya, Ushiroambo, and Lyamba mgongo wards whereas the western part consists of Namonge, Runzewe Mashariki, Runzewe

Magharibi, Igulwa and Ng'anzo. The eastern part of Bukombe has a large number of livestock keeper's as compared to the western part of the district.

As of 2012 only 40.6% of the households have access to improved drinking water sources such as piped water, tube wells and protected springs. The remaining percentage uses non-improved drinking water sources [2,26]. Prevalence of *Salmonella* spp and *Escherichia coli* in raw milk value chain in American Journal of Research Communication.

### Study design and sampling techniques

A cross-sectional study was done to estimate the prevalence of zoonotic *Salmonella* species in water bodies followed by the assessment of antimicrobial resistance of *Salmonella* isolates found in these water bodies in Bukombe district, Tanzania.

### Sample size

The sample size was calculated based on the prevalence of the variables of interest with 0.05 sampling proportion assumed to have safe water with the least desired level of precision

$$n = z^2 \cdot p \cdot q / e^2$$

Where: n= sample size, Z=standard normal deviation, set at 1.96 corresponding to 95% confidence level, p=proportion in the target population estimate; taken at 0.5

$q = 1.0 - p = 0.5$ , e=degree of precision desired, set at 0.05. Therefore the sample size calculated was as follows:

$$n = 1.96^2 \times 0.5 \times 0.5 / 0.05^2 = 384 \text{ samples}$$

Due to the small number of water bodies in the study area, only 240 water samples were examined, of which 120 came from single-purpose water bodies and the other 120 samples from multi-purpose water bodies.

### Sampling and data collection methods

The study was undertaken in 10 wards of Bukombe district namely: Bugelenga, Bukombe, Butinzya, Igulwa, Lyambamgongo, Namonge, Ng'anzo, Runzewe Mashariki, Runzewe Magharibi, and Ushiroambo. In each ward two villages were purposively selected based on the number of livestock, making a total of 20 villages. Four water bodies were selected from each village based on the purpose of each water body (single or multipurpose). Water bodies were checked for evidence of human activities and/or livestock depending on the purpose of the water body (Figure 1). Then three water samples were taken from three different water layers of each water body (at the bottom, middle, and surface).

### Sampling procedure

For the surface water samples, the bottle cap was aseptically removed and the sterile collecting bottle was submerged to a depth of about 20cm with the opening facing slightly upward. The middle and bottom water samples were taken by tying the bottle on a weighted length of a rope by stone or piece of metal of about 500g and then was lowered into the water body to a depth of between one and two meters for the case of middle water sample and to the bottom for the case of bottom water sample. A total of 200ml of water samples were taken in each bottle. The bottles were capped and packed in cool

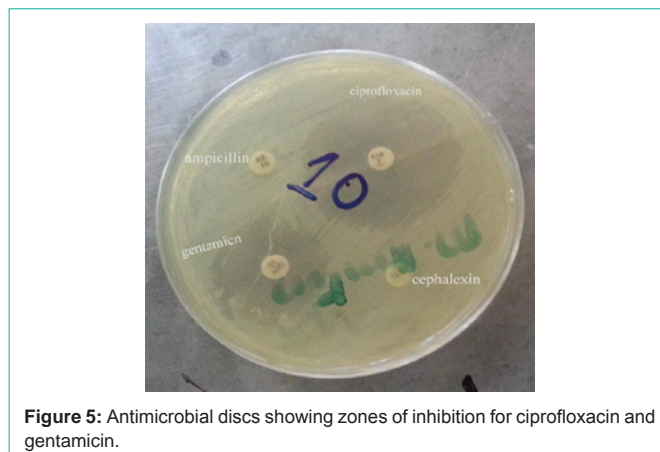


Figure 5: Antimicrobial discs showing zones of inhibition for ciprofloxacin and gentamicin.

boxes. The samples were temporary stored in refrigerator before being transported while in cool boxes to the Microbiology and Molecular Biology Laboratory, Department of Veterinary Microbiology and Biotechnology at Sokoine university of Agriculture in Morogoro for Laboratory analysis. The duration taken from sampling to laboratory analysis was 36 hours (Figure 1).

### Laboratory analysis

**Pre-enrichment of *Salmonella* spp:** Volumes of 25ml of the water sample were mixed with 225ml of buffered peptone water and incubated for 18 hours at 37°C.

**Enrichment of *Salmonella* spp:** One ml of each peptone water culture was introduced separately into 10ml of Rapp port Vassiliadis broth using a sterile syringe and incubated at 42°C for 24h

**Plating on selective medium:** Each selective enrichment broth bottle was well shaken and a loopful from each was streaked onto plates of Xylose Lysine Deoxycholate (XLD) agar and the plates aerobically incubated at 37°C for 24h [26]. The characteristic *Salmonella* spp. colonies on XLD appeared red with black centres. All XLD cultures with characteristic colonies were subcultured on Mac Conkey agar so as to distinguish between lactose fermenters and non-lactose fermenters. The non-lactose fermenting colonies were subcultured on Blood agar to check for swarming property as a way of eliminating *Proteus* species which possess this feature.

**Gram staining:** The slide smears from suspect colonies were prepared and then heat fixed. The smears were flooded with crystal violet and left for 1 minute followed by rinsing with gentle stream of water. Then iodine was poured onto the slide for 1 minute followed by rinsing by alcohol acetone for 3 seconds. The slides were flooded with neutral red for a minute and gently washed with a stream of water. Lastly the prepared slides were observed under microscope at x100. *Salmonella* positive suspects showed gram negative reaction (red/pink colour) with rod shaped organisms (Figure 2).

**Biochemical test:** The suspected isolates were then inoculated on lysine slants and incubated at 37°C overnight; the positive samples had both the slant and the butt purple in colour. Samples positive for *Salmonella* species by the lysine test caused the medium colour to change into purple both at the slant and butt. The test helped to differentiate *Salmonella* species from other *Enterobacteriaceae* such

as *Citrobacter* and *Proteus* spp. As shown in figure 3 below. Other tests such as Triple sugar Iron, Indole and Citrate were done to complement the results (Figure 3).

**Antimicrobial susceptibility testing:** Antimicrobial sensitivity of the *salmonella* isolates were determined by the disc diffusion method on Muller Hinton agar. The bacteria were evenly spread on the medium by streaking the wire loop into all directions and planes. Four classes of antimicrobials namely: penicillins (ampicillin 10 $\mu$ g); cephalosporins (cephalexin 30 $\mu$ g); flouroquinolones (ciprofloxacin 5 $\mu$ g) and aminoglycosides (gentamicin 10 $\mu$ g) which were put at maximum distance from each other to avoid zones of inhibition from coalescing (Figure 4). Isolates were classified as sensitive, intermediate or resistant by measuring diameter of inhibition zone as provided by the Clinical and Laboratory Standards Institute as shown in table 1[27].

#### Molecular confirmation:

**Primers:** The following primers were used; 547-569 Forward-GTG ATC TGA AAT CCA GCT TCA AG, 1055-1078 Reverse-AAG TTT CGC ACT CTC GTT TTT GG (for *S. Enteritidis*), and 139 Forward-GTG AAA TTA TCG CCA CGT TCG GGC AA, 141 Reverse-TCA TCG CAC CGT CAA AGG AAC C (for *S. Typhimurium*) [28].

**Sample preparation and DNA extraction:** Samples of suspected *Salmonella* spp. were taken from individual colonies on the surface of nutrient agar plate, using a sterile plastic loop and resuspended in 4 $\mu$ l of DNA free water. Samples were heated at 100°C for 10min and centrifuged at 1500rpm for 5min to remove cell debris. The supernatant was then transferred into a new tube to be used as a source of DNA.

**DNA amplification:** The PCR reaction mixture contained 5 $\mu$ l of DNA template, 12.5 $\mu$ l of premix, 0.6 $\mu$ l of forward primer, 0.6 $\mu$ l of reverse primer and 6.3 $\mu$ l of DNA free water in the final volume of 25 $\mu$ l. For invA139 forward primer and invA 141 reverse primer (which targeted invA gene with a band size of 284bp), the reaction mixture was incubated at 95°C for 2min, and then cycled 30 times through three steps: denaturing (95°C, 30s), annealing (50°C, 30s), primer extension (72°C, 45s). This was followed by a final extension step at 72°C for 7min. For 547-569 forward primer and 1055-1078 reverse primer (which targeted fliC gene with a band size of 500bp), the PCR was carried out under the following conditions: One initial denaturation cycle was done at 95°C for 5min, followed by 30 cycles of 95°C for 40s, 58°C for 20s, and 72°C for 20s and one final extension cycle of 72°C for 7min.

**Preparation of agarose gel:** The 1.5% agarose gel was prepared by mixing 1.5g of agarose powder with 100ml of TBE (Tris-Borate-EDTA) buffer. This was followed by boiling the gel until became clear, and then it was left to cool. Lastly, Ethidium bromide was added into the gel and poured into the casting tray where the gel was left to solidify.

**Loading of PCR products and Electrophoresis:** 8 $\mu$ l of 50bp DNA marker was loaded into the wells followed by a mixture of 8 $\mu$ l of DNA products and 2 $\mu$ l of blue loading dye. DNA fragments were separated by unidirectional electrophoresis at 110V for 1hr. During loading positive and negative controls were used.

**Table 1:** Classification of *Salmonella* species based on diameter of zone of inhibition in mm.

Drug	Sensitive	Intermediate	Resistant
Ampicillin	$\geq 17$	14-16	$\leq 13$
Cephalexin	$\geq 18$	15-17	$\leq 14$
Ciprofloxacin	$\geq 21$	16-20	$\leq 15$
Gentamycin	$\geq 15$	13-14	$\leq 12$

**Ethical considerations:** The research was conducted according to the Sokoine University of Agriculture Code of Conduct for Research Ethics Section 2.0, Subsection 2.5. The research permit was granted from Faculty Research, Publication and Ethics Committee with reference number SUA/VET/016/25.

**Biosafety measures:** The study was carried out in compliance with Biosafety Level (BSL-2) necessary for all the *Salmonella*, except *S. typhi* where containment equipment and facilities were used during laboratory sample analysis.

**Data management and analysis:** Data entry and validation was carried out using MS-excel 2007 version. Epi Info TM 7 was used for data analysis. Descriptive statistics such as percentage was used to determine the prevalence/level of *Salmonella* spp. Chi-square test was used to compare the prevalence of the bacteria between single and multipurpose water bodies. The significance level for all tests was taken at 5% ( $\alpha = 0.05$ ).

## Results

### Prevalence of zoonotic *Salmonella* spp. from water bodies: Lysine test

Biochemical test using lysine reaction revealed that 10 out of 240 water samples (4.2%) tested positive for *Salmonella* species (Table 2). Out of 120 water samples from multipurpose water bodies seven (5.8%) were positive for *Salmonella* spp. and three (2.5%) of the samples from single purpose water bodies were positive. Half of the positive samples (n=5) were from the eastern part of Bukombe district while the remaining half of the positive samples (n=5) came from the western part of the study area (Table 2).

### The prevalence of zoonotic *Salmonella* spp from water bodies by molecular method: PCR

All positive isolates (n=10) detected by biochemical test (Lysine reaction) were subjected to molecular characterization by PCR by targeting invA and fliC genes for *S. typhimurium* and *S. enteritidis*. Two sets of primers were used, each set targeting one gene. Only 1.3% (n=3) of 240 samples possessed the invA gene hence *S. typhimurium*. None of the isolates contained fliC gene therefore absence of *S. enteritidis*. Of the 120 samples from multipurpose water bodies, 1.6% (n=2) were *S. typhimurium*, where as 0.8% (1/120) sample from single purpose water bodies had invA gene (Figure 4).

### The antimicrobial resistance profile of *Salmonella* spp. from water bodies

All three molecular confirmed *Salmonella typhimurium* isolates (100%) were resistant to penicillin (ampicillin) and cephalosporin (cephalexin), but were sensitive to aminoglycosides (gentamicin) and fluoroquinolones (ciprofloxacin) as shown in figure 5. Gentamicin and ciprofloxacin showed evident zones of inhibition that ranged

**Table 2:** Summary of the prevalence of zoonotic *Salmonella* isolates characterized by biochemical tests.

Ward	Number of samples	positive	Prevalence (%)
Lyambamgongo	24	1	4.1
Iyogelo	24	4	16.7
Bukombe	24	1	4.1
Ushiroambo	24	0	0
RunzeweMagharibi	24	1	4.1
RunzeweMashariki	24	1	4.1
Igulwa	24	0	0
Namonge	24	1	4.1
Ng'anzo	24	1	4.1
Bugelenga	24	0	0
Total	240	10	4.2

**Table 3:** Diameters of zone of inhibition of the three *S. typhimurium* isolates in mm.

Drugs	Isolate1	Isolate 2	Isolate 3
Gentamycin	22	24	21
Cephalexin	0	0	0
Ciprofloxacin	24	38	24
Ampicillin	0	0	0

from 21mm to 38mm (Table 3) (Figure 5).

### Variation of prevalence between the multipurpose and the single purpose water bodies

Both biochemical and molecular analyses revealed a varying prevalence between multipurpose (pond) and single purpose (well) water bodies. Biochemical tests showed that 5.8% (n = 7) of 120 water samples from multipurpose water bodies were positive for *Salmonella* spp. and 2.5% (n=3) of 120 samples from single purpose water bodies were positive. Molecular analyses confirmed that out of 120 samples from multipurpose water bodies, 1.6% (n=2) were positive for *S. typhimurium* and 0.8% (n=1) out of 120 samples from single purpose water bodies were positive for *S. typhimurium*. Statistical comparison between the two types of water bodies showed no significant difference in prevalence (in both biochemical and molecular analysis) ( $P > 0.05$ ) after data analysis by Epi info 7 software.

## Discussion

### The prevalence of zoonotic *Salmonella* isolates from water in Bukombe district

This study has established that the overall prevalence of zoonotic *Salmonella* species from water bodies was 1.3% suggesting that water borne salmonellosis might be a challenge in Bukombe district. This prevalence (1.3%) of zoonotic *Salmonella* observed from water in this study is much lower compared to study done by Mwang'onde, et al. which recorded a prevalence of 99.5% in Dares salaam in Tanzania. The latest study on water in Tanzania reported the prevalence of zoonotic *Salmonella* in northern Tanzania to be 26% [11]. Elsewhere, zoonotic *Salmonella* have been reported in water with the prevalence of 54.2% in Uganda [29], 49.4% in Cameroon [30], 29.6% in Ghana [31]. In Burkina Faso (2% to 31%) [32], and Australia 3% [33,34].

Isolated in Morocco, three serotypes of *S. enterica*: Serotype Blockley (43.8 %), Kentucky (29.8%) and Senftenberg (26.3%).

Detection of *Salmonellae* in the corresponding water samples signifies microbiological contamination of open water sources. The presence of *Salmonella* spp. poses an instant risk to people who drink these waters if untreated [11] and Mwang'onde, et al. One of the sources of *salmonellae* in water from the rural areas of Bukombe may be fecal contamination from animals or humans. However in cities and towns contamination rises from percolation and infiltration of contaminants through the soil under waste disposal sites including pitting latrines [35]. A study done in Kondoa, Tanzania by [36], mentioned limited water supplies, coupled with the lack of hygienic and unreliable water sources, as factors that increase the chance of households using contaminated water during the dry seasons than wet seasons. Higher bacterial concentrations in the water sources are consistent with the scarcity of water due to continual fecal contamination mainly by livestock, wild animals and humans. The lower isolation rate of *Salmonella* spp. in water is probably related to lower incidence of human and animal salmonellosis in the district [37]. The findings of this study indicate that there is a risk for people living in the study area acquiring zoonotic *Salmonella* from the water sources used for domestic purpose. Water scarcity becomes prominent during the dry season which leads to sharing of water sources and consequent contamination of water sources and hence health risks to humans in Tanzania [38].

Although the prevalence (1.3%) of *Salmonella* found in this study appears low, the potential health risk to the public cannot be underrated. This is because carrier animals and humans do switch from one water body to another especially during dry seasons when water becomes scarce; which may lead to contamination of more water bodies. *Salmonella* has been considered a leading cause of water-borne disease outbreaks for the reason that it is commonly isolated from surface waters.

### The antimicrobial resistance profile of *Salmonella* spp. from water bodies

The antimicrobial sensitivity test showed that all *salmonella* isolates (n=3) were sensitive to amino glycosides (gentamicin) and floroquinolones (ciprofloxacin). In contrast they were resistant to penicillin (ampicillin) and cephalosporins (cephalexin). The latest study done in water in northern Tanzania observed a prevalence of 88% of *salmonella* isolates being resistant to one or more antibiotics, with 26% of antibiotic resistant *Salmonella* spp. isolates having the capacity of transferring their resistance traits to *Escherichia coli* [11]. A part from drug resistant water-borne *Salmonella* isolates have been found in other samples; [39], observed resistance of *salmonella* isolates from beef to ampicillin (6.3%) and 100% sensitivity to gentamicin. Elsewhere in Africa, drug resistant *salmonella* have been reported: In Morocco, (49.1%) and displayed resistance to ampicillin, nalidixic acid, sulfonamide compounds and tetracycline [34]. Multiple drug resistant (MDR) *salmonella* strains were also detected in surface water in developed countries [40]. Development of resistant *Salmonella* isolates in water from Bukombe district may have been attributed by improper usage of antibiotics in humans and animals such as over dosage and under dosage of these drugs [41]. Resistant strains eventually find their way into water bodies via human and animal

faeces, or through in appropriate disposal of antibiotics into the environment [42,43], which ultimately reach water bodies through water runoff during rainy season [44]. Moreover, unregulated availability and low cost of these drugs are said to speedup rates of resistance [45]. Isolation of drug resistant *Salmonella* species from water is a public concern, taking into consideration the fact that these water bodies are shared between humans and animals during the dry seasons.

### Variation in prevalence of *Salmonella* spp. isolates between the multipurpose versus the single purpose water bodies

For a while now, studies have labeled water bodies as a “one health” tool (where humans, livestock and wildlife animals interact) which plays an important role in disseminating zoonotic diseases such as salmonellosis. This is due to the fact that humans, livestock and wildlife converge at water sources and contaminate the water with zoonotic pathogens [46]. Water scarcity forces people and animals to use the same water sources for drinking and bathing and laundering, which leads to serious contamination of drinking water and increased risk of zoonotic diseases [47]. It is therefore worth saying that water bodies with high interaction (multipurpose water bodies) have high chances of being contaminated than those with no/limited interaction (single purpose water bodies). This study observed a prevalence of 1.6% of *Salmonella* species in multipurpose water bodies and 0.8% in single purpose water bodies. However, data analysis concluded that the difference in prevalence between the two types of water bodies is not statistically significant. Other studies have demonstrated high prevalence of *Salmonella* isolates in water wells. In Nigeria, three different water sources were contaminated with *Salmonella* enteric serovars with 35.0% being recorded in well water, while 71% of isolates were isolated from uncovered wells [48-51]. The presence of *Salmonella* spp. From well water could have been attributed to contamination via unclean utensils used for fetching water and contaminated surface runoff during rainy season especially because the samples in this study were taken from uncovered wells.

### Conclusions and Recommendations

Based on the results of this study in Bukombe district, the following are conclusions and recommendations to be drawn:

1. The observed prevalence of zoonotic *Salmonella* spp. in water was not as high as reported in other studies, but it is enough to raise public health concern. This calls for research to isolate and identify the zoonotic *Salmonella* species in livestock animals and humans in an effort to establish sources of the contaminant in water bodies. It is, therefore, important for hospitals to consider salmonellosis in differential diagnosis while dealing with diarrheal conditions.
2. The three molecular confirmed *Salmonella typhimurium* isolates showed drug resistance to ampicillin and cephalixin.
3. The study concluded that there was no difference in the prevalence of *Salmonella* species between multi- and single purpose water bodies.
4. There is a need for the authorities responsible for combating drug resistance in humans and animals to carry out educational

campaigns to raise public awareness on causes of antimicrobial resistance and how to minimize it.

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