



Detection and Public Health Risk of *Salmonella* Species Contaminating Different Water Sources in Keffi, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author MOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IGA and AHY managed the analyses of the study. Author AMS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to detect *Salmonella* species and evaluate the public health implications from different water sources in Keffi, Central Nigeria. A total of 100 water samples, 25 each of well, public borehole, tap and sachet water were collected from different locations within the Metropolis and analysed using standard microbiological techniques. Of which 38(38.0%) of the samples were contaminated with *Salmonella* species. The frequency of isolation shows that well water is the most contaminated, 18(72.0%), followed by borehole water, 10(40.0%), tap water, 7(28.0%), while sachet water is the least contaminated with an isolation rate of 3(12.0%). The total bacterial count ranged between $1.0-6.2 \times 10^3$ cfu/ml, while the *Salmonella*/*Shigella* count ranged from $0.2-2.8 \times 10^3$ cfu/ml. The total bacterial count of 6.2×10^3 cfu/ml was recorded for well water, 2.2×10^3 cfu/ml for borehole water, 1.2×10^3 cfu/ml for tap water and 1.0×10^3 cfu/ml for sachet water, while highest *Salmonella*/*Shigella* count of 2.8×10^3 cfu/ml was recorded for well water. The pH for well and

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borehole water were slightly acidic, although that of tap (7.0) and sachet water (7.5) were within permissible limits. The temperature for the water samples were between 25°C–28°C. Meanwhile, turbidity was highest for well water (0.36NTU), in the same vain, total dissolved solid was highest for well water (16.12 mg/l) and lowest for sachet water (0.02 mg/l); while hardness of water was highest amongst the well water samples analysed with a measurement of 48.14 mg/l. The chemical properties of the water samples analysed showed the highest measurements of 6.80 mg/l, 0.78 mg/l and 3.48 mg/l of magnesium, iron and sulphate for well water respectively. Consequently, the presence of microbial contaminants particularly enteric pathogens is indicative of faecal contamination and this can lead to adverse health effects, including gastrointestinal illness and typhoid fever. Therefore, water in Keffi should be properly treated before consumption while boreholes and wells should be dug far away from latrines and septic tanks so as to avoid cross-contamination by faecal materials.

Keywords: Detection; public health; Salmonella; water; Keffi; Nigeria.

1. INTRODUCTION

Salmonella species are Gram-negative facultative anaerobe bacteria and have been isolated from humans, animals, and the environment [1,2,3,4]. *Salmonella* species are among the major pathogenic bacteria in humans as well as in animals, it is the aetiological agent of salmonellosis and typhoid fever [5]. Salmonellosis is an important public health problem causing substantial morbidity and mortality, and thus also has significant economic impact worldwide. On the other hand, the incidence of typhoid fever has decreased in recent years [6], but food-poisoning caused by non-typhoidal salmonella strains has now reached higher proportions in many countries despite improvements in hygiene and sanitation [7,8]. Water is very essential for the existence of humans and other forms of life on earth [9]. Water is the most known and most abundant of all known chemical substances, which occur naturally on the surface of the earth. It is fundamentally important to all plants, animals and man [10]. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs, ocean and wells [11]. Generally, water resource problems are of three main types: too little water, too much water and polluted water [12,13], although the presence of bacteria, viruses, protozoa and helminths can cause water borne diseases [14,15].

Generally, consumption of contaminated water, raw or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors to an outbreak

of *Salmonellosis* in humans [16]. The ubiquity of *Salmonella* isolates creates a persistent contamination hazard in all raw foods [17], while foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized [18,19]. According to reports, every year, a huge number of people suffer from foodborne diseases worldwide due to contaminated food and water consumption [20]. Antibiotics continue to play a very important role in decreasing diseases, illness and/or death associated with bacterial infections [21,22]. Human activities have been largely linked to the emergence of multidrug resistance isolates [23,24]. *Salmonella* species isolated from water samples were resistant to 2 or more antibiotics [9]. Generally, the presence of enteric bacteria in water is a cause for concern due to the potential of water to spread infection within a large over a short period of time. Thus, this research is aimed at the detection and evaluation of public health risk of *Salmonella* species contaminating different water sources meant for human consumption in Keffi metropolis, North Central Nigeria.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The study was carried out in Keffi Metropolis, Nasarawa State. Keffi is located in the middle belt of Nigeria. It is geographically situated on a latitude 8°5'N and longitude 7°52'E. Keffi town is on longitude 850 above sea level and it is the North-West of Lafia, the State capital of Nasarawa State. It is 53 km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria [25]. In the study area, the main source of water includes: Borehole, dug by

individuals and politicians; well, mostly owned by households while some are owned by the community; tap, which is restricted to few locations within the metropolis such as Angwan Lambu, CRDP and GRA areas and rain, which is seasonal). Also, sachet water is readily available within the metropolis for consumption.

2.2 Sample Collection

Water samples were collected from each of the water sources using a sterile glass sample bottle (500 ml). The sampling for well, borehole and tap water was done in five different locations within Keffi metropolis namely: Angwan Lambu, High Court, G.R.A., Liman Abaji and Total Area. Five (5) samples each of borehole, tap and well water were aseptically obtained in these study areas. Well water samples were collected aseptically in sterile bottles tied with a strong string to a piece of metal (about 500 g) as the weight. The bottle cap was first aseptically removed, and the weighted bottle lowered into the well to a depth of about 1-2 m. Thereafter, the bottle was brought up to the surface and covered with the bottle cap aseptically, when no air bubbles is seen inside as described by Akinyemi et al. [26].

Tap and borehole water samples were obtained following the methods of Ibe and Okpelye [27], thus the tap and boreholes were first opened and the water was allowed to run-to-waste for about 2-3 minutes respectively so as to allow any stagnant impurities in the pipe to flush out, after which they were turned off. A piece of cotton wool soaked in methylated spirit was then held with a forcep which was ignited with a lighter to heat-up the tap nozzles until it became unbearably hot to touch so as to prevent contamination from external source. The tap or borehole was then allowed to run continuously for about 1 minute so as to cool the water after which a pre-labelled sample bottle was filled from the gentle flow of water after which the screw cap was carefully replaced. While five different brands of sachet water were also purchased within the metropolis, all the different water samples were properly labelled to capture time of collection, Nigerian National Agency for Food, Drugs Administration and Control (NAFDAC) number and then transported in ice packs to the laboratory immediately after collection for bacteriological analyses.

2.3 Sterilization

The glasswares including conical flasks, Petri dishes and others were sterilized by autoclaving

(moist heat sterilization) at 121°C for 15 minutes, while plastics materials were sterilized in hot air oven (dry heat sterilization).

2.4 Isolation of *Salmonella* species

Isolation of *Salmonella* species was done using the modified method of the Food and Drug Administration of the United States [28]. The various water samples obtained were thoroughly mixed, 1 ml was then retrieved using sterile syringe and transferred into 9 ml of distilled water in a test tube and 10-fold serial dilutions was then made. After which, 0.1 ml from the third diluent (10^{-3} dilution) was inoculated into freshly prepared nutrient agar for the determination of the total bacterial counts accordingly. For the isolation, 1 ml of each water sample was taken from the mixed stock and transferred into 10 ml buffered peptone water, and incubated at 37°C for 24±2 hrs. Afterward, 0.1 ml and 1 ml of pre-enriched aliquots were then transferred into 10 ml of selenite cystine broth. Enrichment samples in the selenite cystine broth were incubated at 37°C for 24 h. Enriched aliquots (ca. 10 µl) was then streaked onto *Salmonella*/*Shigella* agar and brilliant green bile agar and incubated again at 37°C for 24-48 h. All incubations were done under aerobic condition.

2.5 Identification of *Salmonella*

After the incubation, presumptive *Salmonella* species were purified on nutrient agar and were identified using Gram-staining reaction, biochemical tests, and *Salmonella* latex agglutination test as recently described by Adzitey et al. [9].

2.6 Determination of Physicochemical Properties

The physicochemical properties of the water were determined according to procedures outlined in the standard methods for the examination of water and wastewater [29]. Temperature and pH was determined using thermometer and pH meter. Turbidity was determined through the use of a HACH 2100 P Turbidimeter. The dissolved oxygen was measured using dissolved oxygen (DO) meter (Model oxi 197). Total dissolved solid was measured gravimetrically after drying in an oven to a constant weight at 105°C. Sulphate and iron were determined using the photometer (model spectroquant), while magnesium was measured by EDTA titration procedure.

3. RESULTS

The cultural, morphological and biochemical characteristics of the *Salmonella* species isolated from different water sources in Keffi metropolis is presented in Table 1. After incubation, the bacterial colonies were observed to be colourless with traces of black on Salmonella/Shigella Agar and pink-white with brilliant red zones on Brilliant Green Bile Salt agar. The morphology of the isolates showed that *Salmonella* is irregular rod in shape with low convex elevation; it is translucent arranged singly or in pairs. The surface of the colonies is smooth and glistening. The bacterium was confirmed to be Gram negative (pink or redish). The isolate was also confirmed to be catalase positive, indole negative, oxidase negative, methyl red positive and Voges Proskauer negative. The Total Bacterial and Salmonella/Shigella Counts of the isolates were presented in Table 2. The highest bio-load was recorded for well water samples with a total bacterial count of 6.2×10^3 cfu/ml. Meanwhile, the total bacteria counts of borehole water, tap water and sachet water are 2.2×10^3 cfu/ml, 1.2×10^3 cfu/ml and 1.0×10^3 cfu/ml respectively. Similarly, the Salmonella/Shigella

Count was highest for well water with a count of 2.8×10^3 cfu/ml, followed by borehole water with a count of 1.5×10^3 cfu/ml, tap water had 0.8×10^3 cfu/ml and lastly the sachet water is the least contaminated with a count of 0.2×10^3 cfu/ml.

While the rate of isolation of the *Salmonella* species from well, borehole, tap and sachet water from different locations within Keffi metropolis are presented in Table 3. Twenty five [25] samples each of the different water sources were analysed making a total of 100 samples. Of the 25 well water samples analysed, 18 with an isolation frequency of 72.0% were contaminated with *Salmonella*, while 10(40.0%) of the borehole water samples analysed within Keffi metropolis were found to harbour *Salmonella* species. Also, 7(28.0%) of the tap water samples investigated were contaminated with the bacteria and the least contaminated water source is the sachet water with an isolation frequency of 3(12.0%) respectively. Thus 38 samples from the total 100 samples of the four different water sources analysed signifying a total isolation frequency of 38.0% in this study.

Table 1. Cultural, morphological and biochemical characteristics of salmonella specie isolated from different water sources in Keffi metropolis

Cultural characteristics	Morphological characteristics			Biochemical characteristics				Inference	
	Shape	Elevation	Optical feature % colony surface	CAT	IN	OX	MR		VP
G.S									
Colourless colonies with red-black centre on SSA,									
-	I.R	L.C	Translucent Smooth, glistening	+	-	-	+	-	<i>Salmonella</i> spp.
Pink-white colonies Surrounded by brilliant Red zones on BGBS									

Keys: + =Positive, - =Negative, SSA =Salmonella/Shigella Agar, BGBS =Brilliant Green Blue Salt, CAT =Catalase, IN =Indole, OX =Oxidase, MR =Methyl Red, VP =Voges Proskauer, I.R =Irregular Rod, L.C. =Low Convex

Table 2. Total bacterial and Salmonella/Shigella counts of different water samples isolated within Keffi Metropolis ($\times 10^3$ cfu/ml)

Water source	Total bacterial count	Salmonella/Shigella Count
Well water	6.2	2.8
Borehole water	2.2	1.5
Tap water	1.2	0.8
Sachet water	1.0	0.2

Table 3. Rate of isolation of *Salmonella* species isolated from different water sources sampled from different locations in Keffi metropolis

Location	Number of samples per location	Rate of isolation (%)			
		Well water	Borehole water	Tap water	Sachet water
Angwan Lambu	25	5(100.0)	3(60.0)	3(60.0)	1(20.0)
High Court	25	3(60.0)	2(40.0)	2(40.0)	0(0.0)
G.R.A	25	3(60.0)	1(20.0)	0(0.0)	1(20.0)
Liman Abaji	25	4(80.0)	3(60.0)	1(20.0)	0(0.0)
Total Filling Station	25	3(60.0)	1(20.0)	1(20.0)	1(20.0)
Total	100	18(72.0)	10(40.0)	7(28.0)	3(12.0)

Key: G.R.A= Government Reserved Area

Table 4. Physicochemical properties of different water samples analysed in Keffi metropolis

Parameter (Unit)	Well water	Borehole water	Tap water	Sachet water	WHO standard
pH	4.7	5.6	7.0	7.5	6.5–8.5
Temperature (°C)	28	27	26	25	25–30°C
Turbidity (NTU)	0.36	0.04	0.01	0.00	5
Total Dissolved Solid (mg/l)	16.12	8.16	0.88	0.02	500
Hardness (mg/l)	48.14	6.04	3.71	0.80	500
Magnesium (mg/l)	6.80	3.14	2.01	0.92	30
Iron (mg/l)	0.76	0.12	0.04	0.01	0.30
Sulphate (mg/l)	3.48	2.03	0.46	0.12	250

Key: NTU = Nephelometric Turbidity Units

Table 4 showed the physicochemical properties of the water samples. The pH for well water is 4.7, borehole water was 5.6, tap water was 7.0 and sachet water was 7.5. The temperature for the water samples were: well water (28°C) > borehole water (27°C) > tap water (26°C) > sachet water (25°C). Meanwhile, turbidity was highest for well water (0.36NTU), followed by borehole water (0.04 NTU), while the least turbid was the sachet water (0.00NTU). In the same vein, total dissolved solid was highest for well water (16.12 mg/l) and lowest for sachet water (0.02 mg/l); while hardness of water was highest amongst the well water samples analysed with a measurement of 48.14 mg/l, followed by borehole water (6.04 mg/l) and lastly sachet water (0.80 mg/l). The chemical properties of the water samples analysed showed the highest measurements of 6.80 mg/l, 0.78 mg/l and 3.48 mg/l of magnesium, iron and sulphate for well water respectively; while the sachet water had the lowest measurements of 0.92 mg/l, 0.01 mg/l and 0.12 mg/l respectively for magnesium, iron and sulphate.

4. DISCUSSION AND CONCLUSION

4.1 Discussion

In this present study, twenty five (25) samples each of well, rain, borehole and five (5) samples

each of five different brands of sachet water were analysed in order to determine their bacteriological quality as well as the antibiotic susceptibility or resistance of isolated *Salmonella* specie against some common antibiotics. In terms of contamination, well water is the most contaminated with an isolation frequency of 18(72.0%) similar to the findings of Dolejská et al. [30]; followed by borehole and tap water with an isolation frequency of 40.0% and 28.0% respectively in line with frequencies reported by Nwidi et al. [14]. Meanwhile, Sule and his colleagues [31] had demonstrated convincingly that proper chlorination of tap water can prevent the transmission of water-borne diseases but contamination may occur during distribution along rusty-pipes, this perhaps explains why *Salmonella* was identified in this present study. However, the least contaminated water was the sachet water with *Salmonella* isolation frequency of 3(12.0%). This finding corroborates the reports of previous researchers such as Akinyemi et al. (26), Adentunde and Glover [32] and Ahmed et al. [33].

In the same note, the total bacterial count ranged between 1.0–6.2 × 10³ cfu/ml. As suspected, the highest bio-loads were recorded for well water samples and the lowest microbial count was recorded for the sachet water samples. This reinforces an earlier report of Adzitey et al. [9]

that well and river water were the most polluted by microorganisms. Interestingly, several workers such as Adebola [13] and Sule et al. [31] had attributed the presence of *Salmonella* in water to be due to the use of contaminated containers to fetch and store water meant for human consumption and/or use; while the trend of leaving wells open or situating them around sock away had also been implicated as the common source of *Salmonella* contamination for different water sources. The presence of *Salmonella* in sachet water is a further proof of possible adoption of sharp practices by these water manufacturing factories as observed by Oyedeji et al. [34], and also possible connivance by regulatory agencies that are supposed to ensure compliance to good manufacturing practices and stipulated standards. Other authors such as Oluyeye et al. [35] had observed such trends which they implicate as the reason why packaged water may not at all be completely safe for human consumption. Similarly, the sachet water showed a relatively excellent bacteriological quality due to the low *Salmonella/Shigella* count (1.0×10^3 cfu/ml) observed in the water source. This is expected as water from this source appears to be comparably better treated than the former sources which are not treated at all. A cause for concern however is the reported possibility that this sachet water may become spoiled before reaching its final consumers due to its short and limited expiry date (mostly after one month from date of production) as observed by Edeme and Atayese [36]. Also, Oyedeji et al. [34] had reiterated that sachet water that stays for a longer duration may pose health risk to eventual consumers of the water.

More so, the heavy contamination of the well water analysed corresponded with some earlier reports such as those of Nola et al. [37] and Akubuenyi et al. [15] of the poor bacteriological quality and unsuitability of water from this source for human consumption. For instance, Sule and colleagues [31] had earlier noted that groundwater from wells and springs are considered safe only when the guidelines for location, construction and operation are strictly adhered to. They also reported that in most rural areas of developing countries, many ground water supplies are contaminated from sources like seepage pits, septic tanks, privies and cesspools which are located in their vicinities. This corroborates the reports of Adelegan [38] and Da Silva et al. [39]. Another problem that is being cited by many workers is the sitting of drinking water system (wells and boreholes) near

a refuse dumpsite or landfill [40,11]. Consequently, the presence of *Salmonella* specie in the samples analysed is not surprising and perhaps connotes faecal source of contamination as earlier juxtaposed by previous studies including that of Nola et al. [37], Ayandiran et al. [41] and Adzitey et al. [9]. Although this poses considerable hazard to the public in view of the rate at which water can spread infections easily within large populations. Hence, the total bacterial counts revealed that well and borehole rain water are the most contaminated, this may not be far from the fact that this two water sources are the most readily available within the metropolis. Nonetheless, borehole water with a total bacterial count of 2.2×10^3 cfu/ml is unsafe since the World Health Organization had recommended zero coliforms in water meant for human consumption and food preparations [42].

An assessment of the physicochemical properties of the water samples showed that tap (7.0) and sachet water (7.5) were within the WHO standard, while well and borehole water were below WHO limits indicating that they are slightly acidic. This increased acidity could be attributed to the presence of acidic metabolites [43]. The temperature and total dissolved solids of the samples were within WHO guideline values for drinking water. Also, the turbidity of the water samples was within WHO standard with sachet water having no turbidity completely (0.00NTU). Interestingly, turbidity relatively measured the physical or visual observable dirtiness of water resources and is an important indicator of water pollution [44]. However, the relatively high values for well water could be attributed to dumping of solid wastes close to the water source, a phenomenon that is common in Nigeria and Africa at large as noted by Uzoigwe and Agwa [11]. Meanwhile, total hardness is a function of the geology of the area with which the water is associated. It may affect the taste of water as well as influence its lathering ability when used for washing. In this present study, values of the total hardness were within permissible limit and may not constitute any hindrance.

Conversely, magnesium which is usually less abundant in water than sulphate, perhaps due to the fact that magnesium is found in the earth's crust in much lower amounts as demonstrated earlier by Obiri-Danso et al. [45], was highest in this study. Moreover, high concentration of magnesium in drinking water gives unpleasant

taste to the water [42]. The value of iron was within WHO guideline of 0.30 mg/l in all of the water samples analysed except well water with a value of 0.76 mg/l. This increase could also be attributed to weathering of rocks and the presence of corrosive materials in a water body could also contribute to higher iron content in the water [14]. Similarly, the level of sulphate in the water samples studied were below the WHO permissible limits for drinking water.

4.2 Conclusion

This current research has revealed the sanitary conditions and quality of well, borehole, tap and sachet water in Keffi metropolis. Consequently, 72.0% of the well water samples analysed were contaminated by *Salmonella* species, while the high total bacteria count recorded in all the wells, and some public borehole, tap and sachet water samples are a strong indication of the poor sanitary condition within the environment by people that inhabit the sampling areas. Also, the results showed bacterial loads that are above the acceptable standard limits for drinking water set by World Health Organisation. This connotes public health implications. However, most of the physicochemical parameters measured were within permissible limits set by the WHO for drinking water. In view of the above findings, effort should be made to educate the people on the importance of proper siting and construction of well, good hygienic practices in fetching and storing of tap, well and borehole water, and adequate water protection mechanism should be put in place by local health authorities. Sachet water popularly referred to as "pure water" in this part of the world is not at all pure due to their contamination by *Salmonella* spp. as observed in this study, hence, strict regulation needs to be enforced so that the companies can adopt good manufacturing practices during production and packaging. When all these are done, it will go a long way in eradicating the menace of water-borne associated illnesses in the study area in particular and Nigeria in general.

5. RECOMMENDATIONS

- a) Wells should be covered at all times and containers for drawing of water should be properly washed and kept in a tidy environment.
- b) Tap water should be stored in clean containers and covered at all times. There may be need to re-treat the water with chlorine before consumption.
- c) Sachet water should not be consumed once they exceed one month after production or once the water was found to be leaking as bacteria can contaminate the water through the leakage.
- d) Boreholes and wells should be dug far away from latrines so as to avoid cross-contamination by faecal materials.
- e) Adequate treatment method is recommended before these water sources should be consumed in order to avoid water-related diseases due to heavy chemical contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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