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# Occurrence of *Salmonella* Species and other Bacterial Pathogens in Some Water Supplies of Port Harcourt Metropolis, Rivers State, Nigeria

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**A B S T R A C T**

This research work investigated the occurrence of *Salmonella* pathogens in some water sources from Port Harcourt metropolis, Rivers State, Nigeria. Six different water samples, two each from each of the three selected water sources (Borehole, River and Well water) collected from Timber water side River and Choba River; two different wells located in Rumuolumeni, and two different boreholes located in Port Harcourt and were collected and evaluated for percentage occurrence of *Salmonella* species and other waterborne pathogens. The total heterotrophic count was very high ranging from $2.91xl0^{9}$cfu/ml in River water to1.5 x $10^{11}$ cfu/ml in borehole water, while the *Salmonella* count ranged between $1.3x10^{3}$ and $2.9x10^{3}$ cfu/ml for all the water samples. A total of eight different organisms were identified from a combination of results from the colonial morphological and biochemical tests of fifteen suspected isolates. Five out of the fifteen (15) isolates (WS01, 06, 07, 08 and 14) were identified as *Shigella*; *Salmonella* two isolates (WS05 and 10); *Vibrio* (WS09), *Proteus* (WS13), *Escherichia* (WSQ2), *Enterobacter* (WS04), *Klebsiella* (WS13) The water samples were analysed using standard microbiological methods. The result showed that all the water samples evaluated contained more than the recommended level of bacteria for drinking water. The presence of these organisms in the water samples reveals that the water sources were faecally contaminated and not suitable for public use.

## Introduction

The genus *Salmonella* is one of the most common pathogens usually isolated from water and food-producing animals that are the cause of zoonotic infections in humans and animal species. Thus, *Salmonella* infections are one of the major concerns to human health, animals, and the food industry all over the world. According to (Jajere, 2019), *Salmonella enterica* species is the most pathogenic specie in the genus with close to 2,600 serovars that have been already characterized Amongst all these serovars, the *Salmonella enterica* serovars Typhi, Paratyphi A, Paratyphi B, and Paratyphi C can be collectively called Typhoidal *Salmonella*, whereas the other serovars are grouped as

non-typhoidal *Salmonella* (NTS). The typhoidal *Salmonella* strains are known to specifically infect the human host where it causes typhoid fever and paratyphoid fever, both of which are commonly called enteric fever. In many low- and middle-income nations, the major cause of blood stream acquired disease is the *Salmonella* enterica species (Reddy *et al.,* 2010; Deen *et al.,* 2012). According to reports of (Crump *et al.,* 2004), in 2000 typhoid fever caused nearly 21.7 million diseases and 216,000 deaths while paratyphoid fever infections alone led to 5.4 million diseases. Lozano *et al.,* (2012) stated that typhoid and paratyphoid fevers were included in the Global Burden of Disease 2010 (GBD 2010) project, because both were responsible for 12.2 million disability-adjusted life years (Murray *et al.,* 2012) and 190,200 deaths. CDC (2019), reports that the *Salmonella* bacteria cause about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States annually.

Different reports suggests that typhoid fever appears to be more prevalent in sub-Saharan African countries (Breiman *et al.,* 2012) or to that it have not been given the expected attention by governments of the affected states (Crump, 2012). Transmission of typhoidal *Salmonella* is primarily through the consumption of water and food contaminated with human feces. In the low- and middle-income communities the typhoidal *Salmonella* is endemic, consequently the risk of contracting the infection is high due to the prevalence of poor sanitary practices, lack of access to safe food and good quality water sources (Crump *et al.,* 2004). According to 2015 and 2016 figures from the World Health Organization (WHO), some 663 million people, i.e. 9 percent of the world's population do not have access to safe drinking water; while 2.4 billion, representing 40 percent of the world's population lack proper sanitation (hygienic toilet facilities). Although, there have been significant improvements in securing access to clean water, relatively little progress has been made on improving global sanitation in the last decade. Sewage disposal affects people's immediate environments and leads to water-related diseases such as diarrhea that kills 525,000 children under five each year. Back in 2002, the World Health Organization estimated that water-related diseases could kill as many as 135 million people by 2020. In developed countries, most people have flush toilets that take sewage waste quickly and hygienically away from their homes, while in the developing countries the reverse is true. Some of the bacteria that are often reported of polluting our various water bodies include species of *Shigella, Salmonella, Pseudomonas, Escherichia, Vibrio, Proteus, Enterobacter* and *Klebsiella, Staphylococcus, Bacillus, Streptococcus* and *Listeria* (Chitimbar *et al.,* 2012). The other organisms that are found associated with water pollution include *Burkholderia pseudomallel, Cryptosporidium parvum, Giardia lambda,* Norovirus and other viruses and parasitic worms including the *Schistosoma* species (Alayande *et al.,* 2012).

The predominant dependence on water supplied or sourced from bore-holes, rivers and wells for domestic activities including food preparation and drinking and the alarming poor hygiene and or poor waste management across Rivers State, Nigeria, and indeed all developing countries of the world have been blamed for the alarming millions of cases of various bacterial, fungal, protozoa infections and the consequential millions of deaths that are recorded globally each year with some of such bacterial infections being caused by many species of *Salmonella*. Chukwukere (2008) admits that in most developing countries of the world, the average source of drinking water is surface water, which is commonly untreated before use.

People who have access to treated or good drinking water cannot boast of its regularity. Some even drink untreated water from rivers, oceans, rainfall, stream, etc, which have been contaminated. The World Health Organization estimates that 80% of diseases or unavailability of water. Similarly Chukwukere (2008), in her analysis of the microbial contamination of locally packaged sachet water in Port-Harcourt Metropolis, reported contamination of the various water samples by the heavy presence of species of *Klebsiella, Streptococcus, Proteus, Pseudomonas, and Escherichia*, most of which are associated with fecal contamination of their sources of the raw water supply.

Egwari and Aboada (2002) studied the environmental impact on the bacteriological quality of domestic water supplies in Lagos, Nigeria. The result of the study showed the presence of enteric pathogen such as *E.coli*, and various species of *Salmonella, Shigella, Vibrio, Campylobacter*, etc. The result further indicated that shallow wells were more contaminated than deep wells and boreholes. The contamination was higher during periods of heavy rainfall.

According to Kayambo *et al.*, (2006); Lucas and Gilles, (2008), the World Health Organisation estimated that over 1.1 billion people worldwide lack access to adequate supply of clean water. Water sources in Nigeria are not free of bacteria and other microbial contamination. This further emphasizes the urgent need for continued research and the adoption of preventive measures to forestall or control microbial water pollution.

## Materials and Methods

### Sample collection

A total of six water samples were collected from different locations within Port Harcourt metropolis. Different sterile plastic water bottles were used for each of the water supplies (borehole water, well water and river water). The samples were collected as indicated below.

River water: This was collected from two different rivers; (i) Timber water-side river, located along Diobu, Eagle Island Road, Port Harcourt. ii), Choba segment (part of the New Calabar River).

Borehole water: This was collected from the following areas; (i) 11, Elder Harry Wike close, Rumuepirikom by Oro-Ekpo, Port Harcourt. (ii) 360, Ikwerre road, Port Harcourt;

Well water: This was collected from the following areas: (i) A well opposite Ignatius Ajuru University of Education Main gate, Rumuolumeni, Port Harcourt. (ii) A well close to Rumuolumeni Town Hall, Rumuolumeni, Port Harcourt. All the water samples were taken to the Biology Laboratory, Ignatius Ajuru University of Education for analysis.

### Bacteriological Examination of the Water Samples

### Isolation and culture

Total Heterotrophic Bacterial Count (THBC): Nutrient agar was used to enumerate the total heterotrophic bacteria in all water samples. The nutrient agar medium was prepared according to manufacturer's instruction. 0.1 ml of each set of the diluted water sample was inoculated onto sterilized nutrient agar plates and incubated at $37^{o}$ C for 24 hours. Discrete colonies on the plates were counted as total heterotrophic bacteria.

Total Salmonella count (TSC): Salmonella:-Shigella Agar (SSA) medium was used to

culture and isolate Salmonella species while Desoxycholate Citrate Agar (DCA) medium was used for other enteric bacteria. The media were prepared according to manufacturer's instruction.

After enumeration of THBC and TSC, discrete colonies showing different cultural characteristics were picked using a sterile wire loop and sub-cultured onto fresh Nutrient Agar and Desoxycholate Citrate Agar (DCA) plates to obtain pure cultures. Pure colonies from the sub-culture plates were stored in Nutrient Agar slants, prepared in a screw-capped McCartney bottles and incubated for 24hrs at $37^{0C}$.

Morphological and Biochemical Characterisation of isolates: The bacterial isolates were characterized and identified by cultural morphology and biochemical tests as described by Holt *et al.,* (1994).and Cheesebrough (2004).

## Results and Discussion

The result of total heterotrophic bacteria count (THBC), and total *Salmonella* count (TSC) are shown in Table l and figures 1 and 2.

The ugly experiences of contamination of natural water sources used by both animals and human beings by different species of microorganisms have continued to remain a major global threat to the quest for the provision of potable and good quality water. According to Rachna and Disha (2016), the ever increasing population, urbanization and modernization are posing problems of sewage disposal and contamination of natural water sources, posing natural water.

From the investigations of this study, the Timber water-side river and Choba river recorded the highest total heterotrophic bacterial growth ranging from 2.96 x $10^9$cfu/ml to 2.98 x $10^9$cfu/ml, followed by the well water (2.79X$10^9$cfu/ml to 2.87 x $10^9$cfu/ml). The borehole water samples had the least bacterial count ranging from 1.75 x $10^9$cfu/ml to 1.77 x $10^9$cfu/ml. Similarly, the total *Salmonella* species count was highest in the river water with a range of 2.7 x $10^3$ to 2.9 x $10^3$ cfu/ml. The borehole had the least total *Salmonella* species count of 1.3 x$10^3$ to 1.7 x $10^3$ cfu/ml

The total bacterial counts exceeded the maximum permissible microbial limit of the International Commission on Microbiological Specifications for Food and the United States Food and Drug Administration standards.

Using the cultural, morphological charaterisation and biochemical tests, a total of eight different organisms were identified including *Shigella, Salmonella, Pseudomanas, Escherichia, Vibrio, Proteus, Enterobacter* and *Klebsiella* species. Among the eight organisms isolated in this research work, *Shigella* had the highest percentage occurrence of (33.0%) followed by *Salmonella* (20.0%) and *Pseudomonas* (13.3%), while *Escherichia, proteus, Klebsiella, Vibrio* and *Enterobacter* had low counts of 6.7% each.

All the organisms isolated have health implications for man. They include: severe infantile diarrhea caused by *Escherichia*, typhoid fever due to *Salmonella*, Shigellosis from *Shigella*, Cholera from *Vibrio* species, septicemia and neonatal meningitis, wounds and burn infections, nosocomial infections and other opportunistic illnesses resulting from contamination with *Pseudomonas Proteus, Klebsiella* and *Enterobacter* (Talaro, 2008; Brooks *et al.,* 2007; Cheeseborough, 2004; and Ochei *et al.,* 2007). The contamination of water sources by similar organisms have been reported by many

researchers such as (Esomonu *et al.*, 2012; Kumar *et al.*, 2009; Adedeji and Ibrahim, 2011; and Wandili *et al.*, 2011). Although this research identified mainly gram negative bacteria, Bukola *et al.*, 2006; Adedeji and Ibrahim, 2011; and Egwari and Aboaba, 2002 in their different analysis of water samples noted the presence of some gram positive organisms such as *Staphylococcus, Bacillus*, *Streptococcus* and *Listeria* (Table 2 and 3).

**Table.1** Total heterotrophic and Salmonella count of the water samples

| Water Sample | Average no. of colonies for THBC | Average numbers of colonies for Total Salmonella Count(TSC) |
|---|---|---|
| River Water A | 2.9 X $l0^9$cfu/ml | 2.9 x $l0^3$cfu/ml |
| River Water B | 2.8 x $l0^9$cfu/ml | 2.7 x $l0^3$cfu/ml |
| Borehole Water A | 1.8 X $10^9$cfu/ml | 1.3x $l0^3$cfu/ml |
| Borehole Water B | 1.7 x $l0^9$ cfu/ml | 1.7 X $l0^3$cfu/ml |
| Well Water A | 2.9 x $10^9$ cfu/ml | 2.7 x $10^3$ cfu/ml |
| Well Water B | 2.8 x $l0^9$cfu/ml | 2.5 X $l0^3$cfu/ml |

Key

| | | |
|---|---|---|
| River water (i) | - | Timber River, along Diobu-Eagle Island Road, Port Harcourt |
| River water (ii) | - | Choba segment of New Calabar River., Port Harcourt |
| Borehole water (i) | - | No. 11 Eld. Harry Wike Close, by Oro Ekpo, off Ada George/Port Harcourt |
| Borehole water (ii) | - | No. 360 Ikwerre road, Port Harcourt |
| Well water (i) | - | Opposite IAUE main gate, Rumuolumeni, Port Harcourt |
| Well water (ii) | - | Rumuolumeni Community Town Hall, Port Harcourt |

**Table.2** Identification of isolates by biochemical reactions

| S/N | Isolates | Gram reaction | Urease | Citrate | Indole | Coagulas | Oxidase | Catalase | Lactose | Glucose | $H^2S$ | Motility | Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | WSO1 | - | - | - | - | - | - | - | - | A | - | - | *Shigella sp* |
| 2. | WSO2 | - | - | - | + | - | - | - | + | A/G | - | + | *Escherichia sp* |
| 3. | WSO3 | - | - | + | - | - | - | - | - | A/G | + | + | *Salmonella sp* |
| 4. | WSO4 | - | - | + | - | - | - | - | + | A/G | - | + | *Enterobacter sp* |
| 5. | WSO5 | - | - | + | - | - | + | + | - | A/G | - | + | *Pseudomonas sp* |
| 6. | WSO6 | - | - | - | - | - | - | - | - | A | - | - | *Shigella sp* |
| 7. | WSO7 | - | - | - | - | - | - | - | - | A | - | - | *Shigella sp* |
| 8. | WSO8 | - | - | - | + | - | - | - | - | A | - | - | *Shigella sp* |
| 9. | WSO9 | - | - | - | + | - | + | - | - | A | - | + | *Vibrio sp* |
| 10. | WS10 | - | - | + | - | - | + | + | - | A/G | - | + | *Pseudomonas sp* |
| 11. | WS11 | - | - | + | - | - | - | - | - | A/G | + | + | *Salmonella sp* |
| 12. | WS12 | - | - | + | - | - | - | - | - | A/G | + | + | *Salmonella sp* |
| 13. | WS13 | - | + | + | - | - | - | - | - | A/G | + | + | *Proteus sp* |
| 14. | WS14 | - | - | - | - | - | - | - | - | A | - | - | *Shigella sp* |
| 15. | WS15 | - | + | + | - | - | - | - | + | A/G | - | - | *Klebsiella sp* |

Keys: - = Negative Positive
A = Acid production
G = Gas production
$H^2S$ = Hydrogen sulphide
SW water sample

**Table.3** Percentage occurrence of different bacteria species in the water samples

| | Percentage occurrence (%) |
|---|---|
| *Shigella sp.* | 33 |
| *Escherichia sp.* | 6.7 |
| *Salmonella sp.* | 20 |
| *Enterobacter sp.* | 6.7 |
| *Pseudomonas sp.* | 13.3 |
| *Vibrio sp.* | 6.7 |
| *Proteus sp.* | 6.7 |
| *Klebsiella sp.* | 6.7 |

**Fig.1** The occurrence of gram negative bacteria associated with *Salmonella* species in the selected water samples

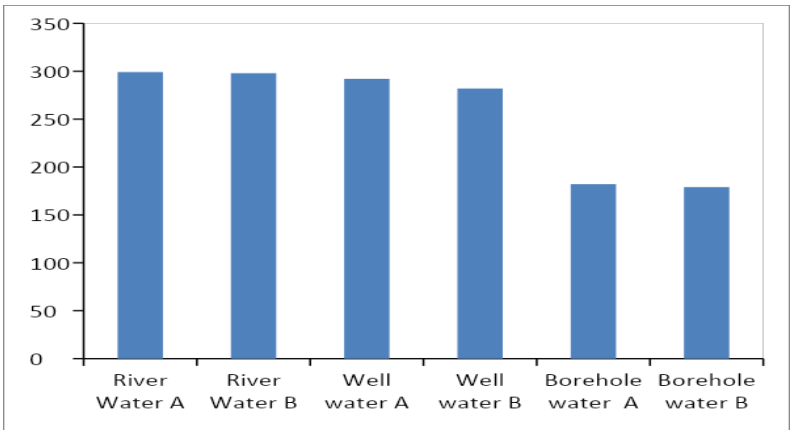
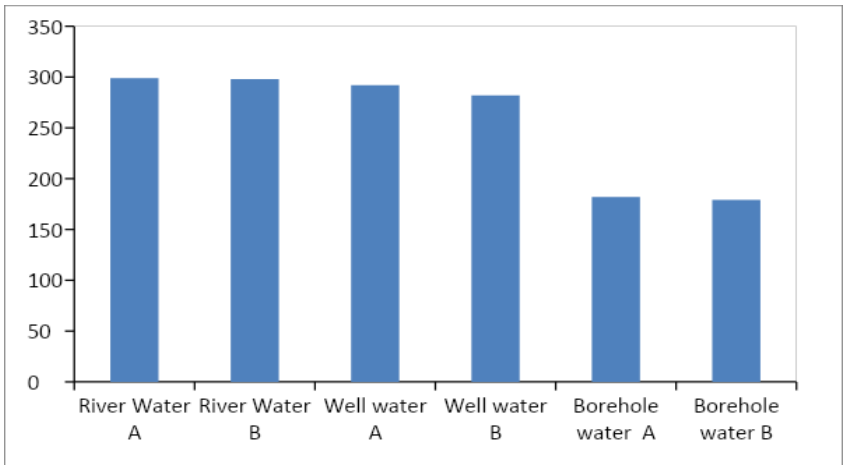



**Fig.2** The occurrence of *Salmonella* species in the selected water samples




Direct defecation, dumping of refuse and the discharge of other untreated wastes into the Timber water-side river and the Choba River was responsible for the high occurrence of heterotrophic bacteria and *Salmonella* species in the water samples. The well water samples from two separate wells within the Port Harcourt metropolis had high bacteria

contamination because of their closeness to septic tanks which is against the 50 feet distance recommended by the World Health Organization. The insensitivity of man with regards to his environment, especially in waste disposal and wastes management, ranging from open defecation, indiscriminate dumping of refuse, discharge of untreated sewage into surface water bodies, to release of untreated chemicals or industrial wastes into the environment have brought upon man different environmental and health challenges.

Amakolonwa (2007) worked on analysis of the microbial quality of commercial bottled water brands in Port-Harcourt metropolis and found the presence of *E. coli* in virtually all bottled water brands. In addition, the *Vibrio* and fungi species were also detected in some of the sampled brands. The total heterotrophic bacteria count ranged from I. l x $l0^3$ to 2.6 x $l0^6$ cfu/ml.

In the analysis of the microbial quality of borehole water from land and swamp locations in parts of Rivers State, Amesi (2007) reported a total heterotrophic count ranged of 1.08 x $l0^6$cfu/ml to 8.0 x $l0^6$cfu/ml for swamp location and 2.5 x $l0^6$cfu/ml to 9.3 x $l0^6$cfu/ml for land location respectively. The bacteria contaminants confirmed were *Bacillus, Flavobacterium, Citrobacter, Pseudomonas, Staphylococcus, Arthrobacter, Escherichia, Micrococcus, Enterobacter* and *Corynebacterium.*

Esonomi *et al.,* (2002) studied enteric pathogens and diarrhea disease potentials of underground tanks and streams water sources in Ahiazu Mbaise, Imo State, Nigeria and found that total heterotrophic bacteria and coliform count ranged between 2.0 x$l0^5$ to 4.8 x $l0^3$ respectively. They identified *E, coli* (with 50% occurrence), *Salmonella* spp. (with 100% occurrence), *Shigella spp.* (100%), *Vibrio spp.* (20%), *Proteus spp.* (30%), *Klebsiella spp.* (80%), *Enterobacter spp.* (50%) and *Streptococcus spp.* (50%) as the contaminating bacteria.

Sewage disposal affects people's immediate environment, and leads to water related illnesses that kills many children under five years old annually. In addition, bacterial contamination of water bodies especially in rivers, seas, renders the aquatic animals (fishes) especially filter feeders, and scavengers unfit for consumption; as their content of such bacteria increases beyond the acceptable standards.

In conclusion the water samples from all the three different water sources analysed contained varying numbers of *Salmonella* species amongst other heterotrophic and coliform bacteria. The *Salmonella* species count occurred in numbers higher than the World Health Organisation recommended limits for drinking water. Clean and potable water should be provided for the people, and in sinking boreholes citizens should adhere to the stipulated standard specifications.

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