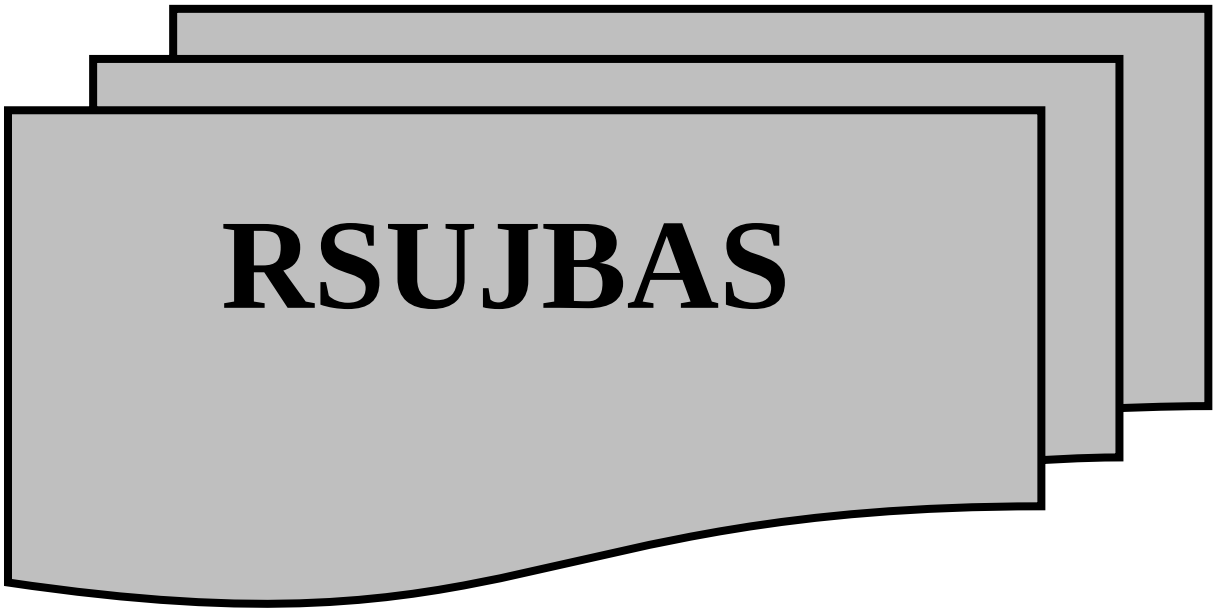
RSU JOURNAL OF BIOLOGY

AND

APPLIED SCIENCES

ISSN: 2811 – 1451



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OCCURRENCE OF SALMONELLA SPECIES IN SOME WATER SUPPLIES OF PORT HARCOURT METROPOLIS, RIVERS STATE, NIGERIA.

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ABSTRACT
This study investigated the occurrence of Salmonella organisms and other enteric pathogens in some water sources from Port Harcourt metropolis, Rivers State, Nigeria. Six different water samples, two from each of the three selected water sources (Borehole, River and Well water) were collected from Timber water-side River and Choba River; two different wells located in Rumuolumeni, and two different boreholes located in Port Harcourt from and evaluated for percentage occurrence of Salmonella species and other enteric pathogens. The water samples were analysed using standard microbiological methods. The total heterotrophic count was very high, ranging from 2.9xl0^{9}cfu/ml in River water to 1.5 x 10^{11} cfu/ml in borehole water, while the Salmonella count from the River water was 3.0 x 10^{9}cfu/ml. A total of eight different organisms were identified colonial morphological and biochemical tests. Five out of the fifteen (15) isolates (WS01, 06, 07, 08 and 14) were identified as Shigella; Salmonella were two isolates (WS05 and 10); Vibrio (WS09), Proteus (WS13), Escherichia (WSQ2), Enterobacter (WS04), Klebsiella (WS13) all occurred once. The result showed that all the water samples evaluated had 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 fiscally contaminated and not suitable for public use.

Key words: Salmonella spp, Enteric pathogens, Water sources, Port Harcourt

INTRODUCTION
The genus, *Salmonella,* represents one of the most common pathogens frequently isolated from water. It is also commonly associated with food handlers who are responsible for human-to-

human transmission of the organism. Thus, *Salmonella* infections are of major concern to public health. The species, *Salmonella enterica* is the most pathogenic species in the genus and there are about 2,600 serovars so far characterized (Jajere, 2019).

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 illnesses 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 remains the case. Some of the bacteria that are often reported polluting our various water bodies include species of *Shigella, Salmonella, Pseudomanas, Escherichia, Vibrio, Proteus, Enterobacter, 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 *Schistosoma* species (Alamance *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 and protozoan 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 illnesses in developing countries are caused by inadequate sanitation, polluted water 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 he associated with fecal contamination of 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.

As cited by Kayambo *et al*. (2006) and 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 bacterial 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.

2. Materials and Methods

2.1 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.

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

(b)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.

(c)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.

2.2 Bacteriological Examination of the Water Samples

2.2.1 Isolation and culture

i) 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 the manufacturer's instruction. 0.1 ml of each set of the diluted water sample was pipetted onto the surface of nutrient agar in agar plates using a 1-ml pipette. A bent glass rod, sterilized over flame was used to spread out the water sample on the agar. The plates were incubated at room temperature (37^{o}C) for 24 hours for growth. Discrete bacterial colonies were counted and converted to colony –forming units per ml. This represented the total heterotrophic bacterial count.

ii) **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 the manufacturer's instruction. After plating out and incubating at 37^{o}C for 24 hours, discrete colonies showing different cultural characteristics were picked using a sterile wire loop and sub-cultured onto fresh Nutrient Agar (*Salmonella* species) and Desoxycholate Citrate Agar (DCA) plates (other enteric bacteria) to obtain pure cultures. Pure colonies from the sub-cultures were stored in Nutrient Agar slants, in screw-capped McCartney bottles and incubated at 37^{0C} for 24 hours.

iii) **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 Cheeseborough (2004).

3. Result

The total heterotrophic bacteria count (THBC) is shown in table 1: Tables 2 and 3 show the biochemical characteristics and percentage occurrence of the isolates, respectively. Figures 1 and 2 show the occurrence of the Salmonella species and the enteric bacteria associated with the water samples, respectively.

Table 1: Total heterotrophic and Salmonella count of the water samples

Water Sample	Average no. of colonies for THBC	Average number 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/ml1.	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 (A) -Timber River, along Diobu-Eagle Island Road, Port Harcourt

River water (B) -Choba segment of New Calabar River., Port Harcourt

Borehole water (A) -No. 11 Eld. Harry Wike Close, by Oro Ekpo, offAda George/ Port Harcourt

Borehole water (B) -No. 360 Ikwerre road, Port Harcourt

Well water (A) Opposite IAUE main gate, Rumuolumeni, Port Harcourt

Well water (B) . Rumuolumeni Community Town Hall, Port Harcourt

Figure 1: The occurrence of Salmonella species in the selected water samples.

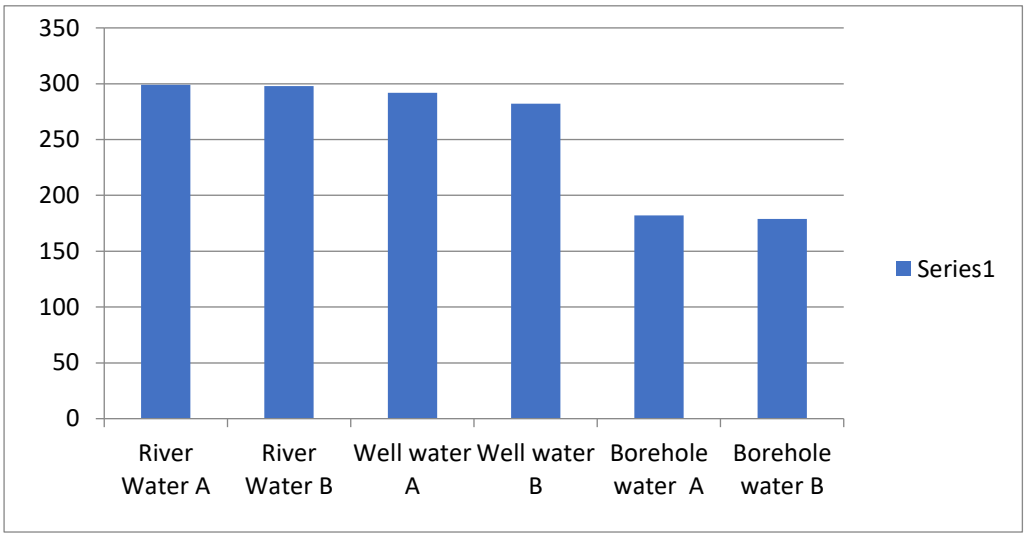

Figure 2: The occurrence of enteric bacteria associated with *Salmonella* species in the selected water samples.

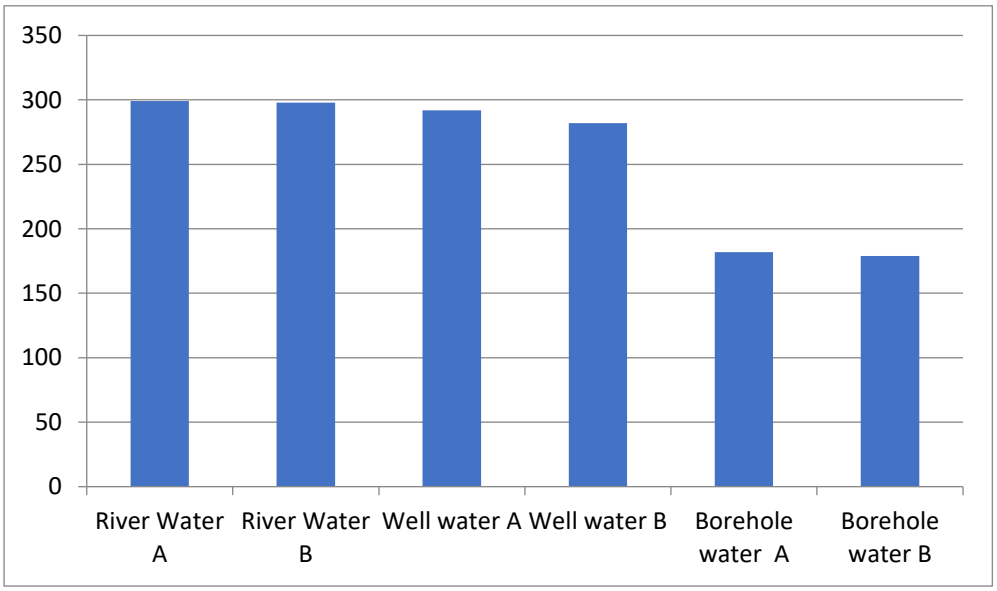

Table 2: Identification of isolates by biochemical reactions

S/N	ISOLATES	GRAM REACTION	UREASE	CITRATE	INDOLE	COAGULAS	OXIDASE	CATALASE	LACTOSE	GLUCOSE	H_2S	MOTILITY	ORGANISM
1.	WSO1	-	-	-	-	-	-	-	-	A	-	-	*Shigellasp*
2.	WSO2	-	-	-	+	-	-	-	+	A/G	-	+	*Escherichia sp*
3.	WSO3	-	-	+	-	-	-	-	-	A/G	+	+	*Salmonella sp*
4.	WSO4	-	-	+	-	-	-	-	+	A/G	-	+	*Enterobactersp*
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
WS = Water sample

Table 3: Percentage occurrence of the different enteric bacterial species in the water samples

Bacteria	Percentage occurrence (%)
Sh*igella sp.*	3.33
Escherichia sp.	6.7
Salmonella sp.	20.0
Enterobacter sp.	6.7
Pseudomonas sp.	13.3
Vibrio sp.	6.7
Proteus sp.	6.7
Klebsiella sp.	6.7

DISCUSSION

The ugly experiences of contamination of natural water sources used by both animals and humans 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 pose problems of sewage disposal and contamination of natural water sources.

From the findings of this study, the Timber water-side river and Choba river recorded the highest total heterotrophic bacterial count which ranged from 2.96 109 cfu/ml to 3.0 x 10^9 cfu/ml, followed by the well water (2.8 x 10^9 cfu/ml to 2.9 x 10^9 cfu/ml). The borehole water samples had the least bacterial count ranging from 1.75 x 10^9cfu/ml to 1.77 x 10^9cfu/ml. Similarly, the total *Salmonella* species count was highest in the river water with a range of 2.7 x 10^3 to 3.0 x 10^3 cfu/ml. The borehole had the least total *Salmonella* species count of 1.3 x10^3 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 and morphological characterisation 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 (33.0%), followed by *Salmonella* (20.0%) and *Pseudomonas* (13.3%)*; Escherichia, proteus, Klebsiella, Vibrio* and *Enterobacter species* had low counts of 6.7% each.

All the organisms isolated have health implications for man. They include: severe infantile diarrhea caused by enteropathogenic *Escherichia coli* (EEC); typhoid fever due to *Salmonella* species; Shigellosis by *Shigella* species; Cholera by *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*. (Cheeseborough, 2004, Brooks *et al*., 2007; Ochei *et al*., 2007, and Talaro, 2008;).The contamination of water sources by similar organisms have been reported by many researchers (Kumar *et al.,* 2009; Adedeji and Ibrahim, 2011; and Wandili et *al.,* 2011 Esomonu *et al.,* 2012;).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* species.

Direct defecation, dumping of refuse and the discharge of other untreated wastes into the Timber water-side river and the Choba river were 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 bacterial 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 management, ranging from open defecation, indiscriminate dumping of refuse, discharge of untreated sewage into surface water bodies, to the 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 species of fungi were also detected in some of the sampled brands. The total heterotrophic bacteria count ranged from I.lxl0^{3}to 2.6xl0^{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 range of 1.08xl0^{6} cfu/ml to 8.0xl0^{6} cfu/ml for swamp location and 2.5xl0^{6} cfu/ml to 9.3xl0^{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 tank and stream-water sources in Ahiazu Mbaise, Imo State, Nigeria and found that total heterotrophic bacteria and coliform count ranged from 2.0xl0^{1-} to 4.8 x l0^{3} respectively. They identified *E, coli* (50% occurrence), *Salmonella* spp. (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 kill many children under five years old annually. In addition, bacterial contamination of water bodies especially rivers and seas, renders the aquatic animals especially filter feeders, and scavengers unfit for consumption; their bacterial had increases beyond the acceptable standards.

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