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# Evaluation of Salmonella Species in Water Sources in Two Local Government Areas of Anambra State

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**Abstract**

Microbial quality of water using *Salmonella* species in water sources was investigated in two Local Government Areas in Anambra State, between the months of September and December, 2013. A total of 220 water samples were analyzed using Bacteriological Analytic method and membrane filtration method. Antimicrobial Susceptibility test by disc diffusion method and serotyping was done using slide agglutination methods with polyvalent antiserum. *Salmonella species* was most prevalent (35.0%) in Reservoir water samples and least prevalent (7.45) in Borehole-water samples examined. *Salmonella typhi* had the highest prevalence rate of (40.05), *Salmonella enteric* (92.80%), *Salmonella paratyphoid A* (23.05), and Salmonella paratyphoid C least prevalent (9.43%). Antibiotic susceptibility test showed that 24 *Salmonella species* (44.46%) were sensitive to10ug Ciproflaxin while Penicillin (30mg) had the highest resistance rate of 54 (100%). This study portrays the diversity of *Salmonella species* and indicator organisms in water sources in the study area. There is need therefore to put in place appropriate intervention strategies to control the spread of *Salmonella bacilli* from these sources to human population.

**Keywords**: *Salmonella species*; Water sources; Two Local government areas of anambra state

## Introduction

Water is an essential part of human nutrition, both directly as drinking water or indirectly as constituent of food; in addition to various other applications in daily life. Water is not only essential for life, it also remains a most important vehicle of transmitting disease and infant mortality in many developing countries and even in technologically more advanced countries [1]. Good quality water is odorless, colourless, tasteless, and free from fecal pollution [2]. However, it is estimated that about 1.2billion individuals world-wide do not have access to potable water. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities that depend on non-public water supply system [3]. Increase in human population has exerted an enormous pressure on the provision of safe drinking water in developing countries [4]. To curb this health problem, bottled water was introduced but only individuals who have a good financial status can afford these products. Low income earners are left with no option but to consume sachet water, borehole water, wells, reservoirs, surface waters that are usually not free of microbial contaminants [5]. The potential ability of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well document in many countries at all levels of economic development [6]. Water pollution has continued to create negative impacts on health and economic development in Nigeria [7]. High incidence of childhood diarrhoea, helminthiasis, trachoma, typhoid fever and the overall high mortality rates are associated with poor environmental sanitation [8]. Therefore, this study evaluates Salmonella from drinking water source to determine the portability of the water.

*Salmonella* is a genus of rod-shape, gram negative, non spore-forming, predominantly motile Enterobacteria with diameters around 0.7 to 1.5µm, lengths from 2 to 5µm and flagella that move in all directions. They are chemo organotrophs, obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes. Most species produce hydrogen sulfide [9] which can readily be detected by growing them on media containing ferrous sulfate. Most isolates exist in two phases: a motile phase (I) and a non motile phase (II). Cultures that are non-motile upon primary culture may be switched to the motile phase. *Salmonella* is closely related to the Escherichia genus and are found worldwide in cold and warm blooded animals (including humans), and in typhoid fever, paratyphoid fever and food borne illness [10].

*Salmonellosis* is a food borne infection caused by a group of gram-negative motile rods belonging to the family of Enterobacteriaceae. The genus *Samonella* which causes *Salmonellosis* comprises several

species of which *Salmonella typhi* and *Salmonella paratyphi* are the main species pathogenic to man. The species *Salmonella typhimurium* is the common cause of gastroenteritis, while Salmonella chloreraesus is responsible for septicemia. Salmonellosis is associated with several symptoms including diarrhoea, vomiting, nausea, abdominal pain and fever. Untreated cases could become chronic or fatal. The portal of entry for Salmonellosis is through ingestion of contaminated food and/or water contaminated with animal or human faeces. The infection is commonly treated with antibiotics such as Ampicillin, Chloramphenicol, Trimethoprim, Sulpha-methaxazole, and Cephalosporin. Salmonellosis can be controlled or prevented by adequate supervision of food and water, as well as improved personal hygiene [11]. As a result of the serious consequences and threats to health and economic development which water pollution poses, this work is therefore embarked upon to ascertain the microbial quality of drinking water with the following aim and objectives.

## Aim

To evaluate the microbial water quality of various domestic water sources by the presence of *Salmonella species.*

## Specific Objectives

i. To determine the bacteriological quality of ground water and surface water used for drinking in Nnokwa, Alor, Nnobi, Abatete and Oraukwu.

ii. To ascertain the prevalence of different *salmonella species* in different water sources.

iii. To isolate and identify the various *salmonella serovas* found in the water sources.

iv. To determine the susceptibility pattern of *salmonella* isolates from different water sources.

v. To compare the water quality between the areas and their sources.

## Materials and Method

**Study area:** This work was carried out in some selected communities in Idemili North and South LGA.

**Methods of sample collection:** A total of 220 water samples were collected, 50 samples were collected each from these water sources (well, borehole, reservoirs and surface water) and 20 controls (sterile water) between the months of September and December 2013.

**Storage and transport:** The water samples were immediately stored in an ice box at low temperature and immediately transported to the laboratory. Samples were analyzed within 6 hours of collection [12].

**Analysis of sample:** Two methods namely;

a) Membrane filtration method [12]

b) And Dilution Method of standard analytical protocol [13] were used for sample analysis.

### Isolation of *salmonella*

**Membrane filtration method**

**a) Day 1 - Pre-enrichment (Non-selective enrichment)**: Water samples (100ml) were filtered through a sterile (0.45µm) milipore membrane filter. The membrane filter was lifted with a blunt edge forceps and transferred into 90ml of buffered peptone water. These were gently mixed and incubated for overnight at 37 0C.

**b) Day 2 – Selective enrichment I & II method:** A 1ml volume of the pre-enrichment agar was transferred with a pipette into 10ml Tetrathionate broth and 0.1ml of the pre-enrichment was transferred with a pipette into 10ml Rappaport-Vassiliadis Soy Peptone (RVS) broth was incubated at 37 0C.

**c) Day 3 - Selective plating method:** A 10µl loopful of culture each from the tube 1 and tube 2 were streaked on selective agar Chromagar *Salmonella* (France) and Miller Mallinson agar (Switzerland) respectively and incubated at 37 0C overnight. Colonies of the suspected *Salmonella* species on chrom agar *salmonella* appeared as mauve colonies and grey black on miller malison agar. Presumptive *Salmonella* isolates were purified on MacConkey agar plates. Suspected colonies of salmonella were streaked onto MacConkey agar for purification and to achieve single colony. The plates were incubated at 37 0C for 24hrs.

**Dilution method**

**a) Pre-enrichment (non-selective enrichment) method**: Tubes are arranged in three rows (10ml of 3x, 5ml of 3x and 10ml of 1x) of five tubes each. To the first row - 10ml of 3x universal pre-enrichment broth: 20ml of water sample was inoculated to each of the five tubes of 10ml of 3x universal pre-enrichment broth. To the second row - 5ml of 3x universal pre-enrichment broth: 10ml of water sample was inoculated to each of the five tubes containing 5ml of 3x UPB. To the 3rd row - 10ml of 1x universal pre-enrichment broth: 1 ml of water sample was inoculated into each of the five tubes of 10ml of 1x UPB. All the tubes were incubated at 37 0C for 24±2 hrs.

**b) Selective enrichment:** From each tube with growth 1.0ml of the pre-enrichment broth Selenite Cystine broth was prepared according to manufacturer's instruction, was transferred into 10ml of selenite cystine broth. The tubes were shaken and incubated at 37 0C 24+2hrs.

**c) Selective plating:** 3ml loopful (10µL) of the incubated medium was streaked on Chromagar *Salmonella* and Miller-Mallinson agar (MM agar).These were incubated at 37 0C for 24hrs. Plates were examined for the presence of suspected colonies *Salmonella*. Suspected colonies were streaked onto tryptic soya agar for purification, biochemical and serological identification. The plates were incubated at 37 0C for 24 hrs.

### Biochemical test for identification of isolates

**a) Triple sugar iron agar (TSIA)**

**Procedure:** A small amount of growth was harvested with a



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sterile inoculating needle, a single stab was made into the butt of the tube and the surface of the agar slant lightly inoculated. Tubes were incubated under aerobic conditions at 36+1 0C with the caps loosened. Tubes were examined for acid butt and alkaline slant, gas production and hydrogen sulphide production at 24hrs, 48hrs for 5-7 days (unless H2S production occurred sooner) [13].

**b) Lysine iron agar (LIA)**

**Procedure:** A small amount of growth was harvested with a sterile inoculating needle, a single stab was made into the butt of the tube and the surface of the agar slant was lightly inoculated. Tubes were incubated under aerobic conditions at 36+1 0C with cap loosened. Tubes were examined for Hydrogen sulphide production.

**c) Simmons citrate utilization test**

**Procedure:** A small amount of growth was harvested with a sterile inoculating loop. The surface of the agar slant was lightly inoculated. Tubes were incubated under aerobic conditions at 36+1 0C with caps loosened. Tubes were examined for change in colour from blue to green and result recorded at 24hrs, 4hrs for 5-7 days.

**d) Motility -indole-urea (MIU)**

**Procedure:** A small amount of growth was harvested with a sterile needle. A single stab was made into the tube of MIU agar. The stab was made straight into the agar and stopped approximately Icm from the bottom of the tube. And Kovac's reagent paper attached at the neck of the test tube. Tubes were incubated under aerobic conditions at 36+1 0C with caps loosened. Tubes were examined for motility, indole production seen on Kovac's reagent paper and urease production and results recorded following over night (18-24hrs) incubation [14].

**e) Sugar fermentation test**

**Procedure:** Bijou bottles containing about 6ml aliquot of peptone water medium, bromothymol blue indicator and inverted durham tube were sterilized at 121 0C for 15 minutes in the autoclave. One percent of the sugar prepared by dissolving 1g of the sugar in 10ml of water and sterilized by steaming for 30 minutes. The bottles were allowed to cool. Sugar solution of 0.3ml volumes each were aseptically dispensed into bijou bottles containing the basal medium and inoculated with 24hrs culture of each of the isolate and incubated at 37 0C. The production of acid and gas were observed after 24hrs. Acid production was indicated by change in colour of the medium from red to yellow while gas production was indicated by the presence of bubble in the durham tube.

**f) Oxidase test**

**Procedure:** Three drops of freshly prepared oxidase reagent were place on to a paper in a petri disc. With sterile platinum wire loop, some of the growths were smear on the prepared filter paper. Appearance of deep blue purple colour within 10 seconds was regards as positive reaction. Salmonella is oxidase negative, *pseudoman eroginosa* oxidase positive.

## Results

In Table 1, *Salmonella typhi* had the most occurrences both in wells, boreholes, reservoirs and surface water using B.A.M method. In Table 2, *Salmonella typhi* had the most occurrence both in wells, boreholes, reservoirs and surface water using Membrane Filtration

### Antibiotic susceptibility test of *salmonella* isolates

The sensitivity of the pure culture of bacterial isolates to different antibiotics was determined using the Kirby bauer diffusion technique and interpreted based on the guidelines of the clinical and laboratory standards [15]. Commercially prepared antimicrobial disc (Optum Diagnostic Nigeria) were used and it contained the following antibacterial agents. Ciprofloxacin (10mcg), Streptomycin (30ug), Preflacine (10ug), Septrin (30ug), Ampicilin (30ug), Tarivid (10ug), Penicillin (10mcg), Gentamycin (10mcg), Augmentin (30mcg), Nalidic Acid (30mcg). Muller Hinton agar plates prepared according to manufacturer's instructions were used. Commercially prepared antimicrobial disc were placed in the Muller Hinton agar plates after seeding the media with suspected *Salmonella* isolates. These were incubated at 37 0C for 24hrs after which zones of clearance or inhibition were noted and recorded accordingly in mm.

### Serological identification of *Salmonella*

I. Widal kit (cromatestwidal) was used to support the tentative identification of isolates as member of *salmonella* specie. Widal test is a serological technique which test for the presence of *Salmonella* antibodies. It is a screening test used in diagnosing typhoid and paratyphoid fever [12].

**II. Serotyping of salmonella using polyvalent antiserum method**

Three separate drops of normal saline were placed on a clean glass slide using a wire loop. Growth from the overnight culture plate of tryptic Soy Agar was emulsified in each separate drop of normal Saline. A loop of Salmonella Polyvalent 'O' (PSO) anti-serum was then mixed with the first drop of suspension of normal saline while a loop ful of *Salmonella* Polyvalent 'H' antiserum was mixed with the second drop of normal saline. To the third drop (control), one loopful of normal Saline was added. All bacterial Suspension was mixed using a sterile wire loop. The slide was gently tilt back and front for one minute and observed for agglutination under normal light condition. Presence of agglutination indicates positive reaction.

## Statistical Analysis

Statistical Analysis of results was done using Anova, T-test, Chi-square. Level of significant was set at 95% confidence limit and 0.05 levels respectively. Statistical Package for Social Science (SPSS) Software Version 16 was used for evaluation.

Method. In Table 3 below, *Salmonella typhi* had the highest frequency of 5(38.5%) while *Salmonella paratyphi* C has the least frequency of 1(7.7%). In Table (4-10) below, well water at Nnokwa had the highest *Salmonella Spp.* of 80(8.03%), Alor had the highest *Salmonella Spp.* in surface water, borehole and reservoir and Nnobi had no *Salmonella Spp.* in borehole water.



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**Table 1:** Different species of *Salmonella* isolated from various water samples using bacteriological analytical method (B.A.M).

| Microorganism (cfu/100ml) | Wells | Boreholes | Reserviors | Surface Water | Total |
|---|---|---|---|---|---|
| *Salmonella typhi* | 4 | 2 | 5 | 5 | 16 |
| *Salmonella paratyphi A* | 2 | 0 | 3 | 3 | 8 |
| *Salmonella paratyphi C* | 1 | 0 | 0 | 3 | 4 |
| *Salmonella enterica* | 3 | 2 | 3 | 5 | 13 |
| Total | 10 | 4 | 11 | 16 | 41 |

**Table 2:** Different species of *Salmonella* isolated from various water samples using membrane filtration method.

| Microorganism (Cfu/100ml) | Wells | Boreholes | Reserviors | Surface Water | Total |
|---|---|---|---|---|---|
| *Salmonella typhi* | 2 | 0 | 3 | 0 | 5 |
| *Salmonella paratyphi A* | 0 | 0 | 3 | 1 | 4 |
| *Salmonella paratyphi C* | 0 | 0 | 0 | 1 | 1 |
| *Salmonella enterica* | 1 | 0 | 2 | 0 | 3 |
| Total | 3 | 0 | 8 | 2 | 13 |

**Table 3:** Frequency of different *Salmonella* species isolated from various water samples using membrane filtration method.

| Microorganism (Cfu/100ml) | Frequency | Percentage (%) |
|---|---|---|
| *Salmonella typhi* | 5 | 38.5 |
| *Salmonella paratyphi A* | 4 | 30.8 |
| *Salmonella paratyphi C* | 1 | 7.7 |
| *Salmonella enterica* | 3 | 23.1 |
| Total | 13 | 100 |

**Table 4:** Prevalence of total Salmonella spp. isolated from water bodies in Idemili North (Oraukwu and Abatete)

| Sources | *Salmonella typhi* (Cfu/100ml) | *Salmonella paratyphi A* (Cfu/100ml) | *Salmonella paratyphi C* (cfu/100ml) | *Salmonella enteric* (cfu/100ml) | Total |
|---|---|---|---|---|---|
| **Oraukwu** | | | | | |
| Surface water | 17(20.0) | 23(20.0) | 28(20.1) | 2(20.0) | 70(42.9) |
| Borehole | 2(16.7) | 0 | 4(22.2) | 8(20.0) | 14(8.59) |
| Reservior | 30(25.0) | 27(50.0) | 0 | 0 | 57(34.97) |
| **Abatete** | | | | | |
| Borehole | 0 | 2(20.0) | 0 | 0 | 2(1.23) |
| Reservior | 20(25.0) | 0 | 0 | 0 | 20(12.3) |

**Table 5:** Prevalence *Salmonella spp* isolated from water bodies in Idemili South (Nnobi, Nnokwa and Alor).

| Sources | *Salmonella typhi* (CFU/100ml) | *Salmonella paratyphi A* (CFU/100ml) | *Salmonella paratyphi C* (CFU/100ml) | *Salmonella enterica* (CFU/100ml) | Total |
|---|---|---|---|---|---|
| **Nnobi** | | | | | |
| Surface water | 0 | 0 | 5(16.7) | 4(18.2) | 9(0.9) |
| Reservior | 6(31.6) | 0 | 0 | 17(34.0) | 23(2.31) |
| Borehole | 0 | 0 | 0 | 0 | 0 |
| **Alor** | | | | | |
| Surface water | 7(17.5) | 6(17.1) | 2(20.0) | 3(15.0) | 18(1.81) |
| Borehole | 39(50.0) | 15(50.0) | 0 | 0 | 54(5.4) |
| Reservior | 50(33.3) | 10(33.3) | 0 | 0 | 60(6.02) |
| **Nnokwa** | | | | | |
| Well | 34(16.9) | 5(16.1) | 8(16.0) | 33(16.5) | 80(8.03) |



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**Table 6:** Comparism of *Salmonella spp* found in surface water in Idemili South and Idemili north LGA of anambra state.

| Organisms | Idemili South and North | (CFU/100ml) | MEAN±STD | P-Value |
|---|---|---|---|---|
| *Salmonella typhi* | Surface water south | 40 | 1.275±0.452 | |
| | Surface water north | 85 | 8.831±1.673 | 0.000* |
| *Salmonella taratyphi C* | Surface water south | 10 | 5.000±0 | |
| | Surface water north | 139 | 19.244±1.6 | 0.000* |
| *Salmonella paratyphi A* | Surface water south | 35 | 3.428±0.502 | |
| | Surface water north | 114 | 14.373±1.79 | 0.000* |
| *Salmonella enterica* | Surface water south | 20 | 6.000±0 | |
| | Surface water north | 0 | 0.000±0 | 0.000* |

P-Value is significant if ($p<0.05$) and not significant if ($p>0.05$)

**KEY:**

* = Significant

CFU = Colony forming unit

N = Number of organism

STD = Standard Deviation

**Table 7:** Comparism of *Salmonella spp* found in surface water in Nnobi and Alor in Idemili south LGA of anambra state surface waters.

| Organism | Name of Community | (CFU/100ml) | MEAN±STD | P-Value | T-Value |
|---|---|---|---|---|---|
| Salmonella typhi | Nnobi | 0 | 0.000±0 | | |
| | Alor | 40 | 1.250±0.043 | 0.000* | - |
| Salmonella paratyphi C | Nnobi | 0 | 0.000±0 | | - |
| | Alor | 35 | 3.428±0.502 | 0.000* | |
| Salmonella paratyphi A | Nnobi | 30 | 5.333±0.479 | | -10.897 |
| | Alor | 10 | 7.000±0 | 0.000* | |
| Salmonella enterica | Nnobi | 10 | 7.000±0 | 0.000* | -19.039 |
| | Alor | 20 | 8.000±0 | | |

P-Value is significant if ($p<0.05$) and not significant if ($p>0.05$)

**KEY:**

* = Significant

CFU = Colony forming unit

N = Number of organism

STD = Standard Deviation

**Table 8:** Comparism of Salmonella spp found in reservoirs in Nnobi and Alor in idemili south LGA of anambra state reservoirs

| Organism | Name of Community | CFU/100ml | MEAN±STD | T-Value | P-Value |
|---|---|---|---|---|---|
| *Salmonella typhi* | Nnobi | 19 | 1.000±0 | | |
| | Alor | 150 | 2.000±0 | 21.840 | 0.000* |
| *Salmonella paratyphi C* | Nnobi | 0 | 0.000±0 | | |
| | Alor | 19 | 7.000±0 | | 0.000* |
| *Salmonella paratyphi A* | Nnobi | 0 | 0.000±0 | | |
| | Alor | 0 | 0.000±0 | | 0.000* |
| *Salmonella enterica* | Nnobi | 50 | 5.137±0.775 | 47.332 | |
| | Alor | 0 | 0.000±0 | | 0.000* |

P-Value is significant if ($p<0.05$) and not significant if ($p>0.05$)

KEY:

* = Significant

CFU = Colony forming unit

N = Number of organism

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**Table 9:** Comparism of Salmonella spp found in bore holes in Abatete and Oraukwu in Idemili North LGA of anambra state bore hole

| Organism | Name of Community | (CFU/100ml) | MEAN±STD | P-Value |
|---|---|---|---|---|
| Salmonella typhi | Abatete | 0 | 0.000±0 | |
| | Oraukwu | 10 | 2.000±0 | 0.000* |
| Salmonella paratyphi C | Abatete | 10 | 0.909±0.301 | 0.000* |
| | Oraukwu | 0 | 0.000±0 | |
| Salmonella paratyphi A | Abatete | 0 | 0.000±0 | |
| | Oraukwu | 18 | 3.000±0 | 0.000* |
| Salmonella enterica | Abatete | 0 | 0.000±0 | 0.000* |
| | Oraukwu | 40 | 0.000±0 | |

P-Value is significant if ($p<0.05$) and not significant if ($p>0.05$)

KEY:

* =Significant

CFU =Colony forming unit

N =Number of organism

STD =Standard Deviation

**Table 10:** Comparism of conventional and membrane methods used for isolation of Salmonalle spp. from waters bodies.

| *Salmonella Spp.* | Method of Isolation | *Salmonalle* Isolated | MEAN±STD | P-value |
|---|---|---|---|---|
| *Salmonella typhi* | Conventional method | 682 | 7.89±6.82 | |
| | Membrane Method | 124 | 3.18±1.14 | 0.000* |
| *Salmonella paratyphi A* | Conventional method | 263 | 19.45±2.01 | |
| | Membrane method | 158 | 7.79±1.24 | 0.000* |
| *Salmonella paratyphi C* | Conventional method | 95 | 25.00±1.17 | |
| | Membrane method | 7 | 7.00±0.00 | 0.000* |
| *Salmonella enterica* | Conventional method | 315 | 32.12±4.20 | |
| | Membrane method | 140 | 4.57±0.73 | 0.000* |

P-Value is significant if ($p<0.05$) and not significant if ($p>0.05$)

* Significant

**Table 11:** Condition of water sampled sources.

| Variable Sources of Water | Frequency | Prevalence (%) | P -Value |
|---|---|---|---|
| Borehole | 379 | 49.34 | 0.000* |
| Other sources | 101 | 64.35 | |
| **Storage mode of the borehole** | | | |
| Plastic overhead tank | 40 | 80 | 0.000* |
| Metal overhead tank | 10 | 20 | |
| **Regular washing of tanks** | | | |
| Yes | 32 | 68 | 0.000* |
| No | 48 | 90.5 | |
| **How often do you wash tank** | | | |
| Every 1-3 Weeks | 6 | 30 | 0.000* |
| 4-6 Weeks | 26 | | |
| 6 Weeks and above | 48 | 92.5 | |
| **Depth of the borehole** | | | |



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| | | | |
|---|---|---|---|
| Less than 350ft | 35 | 83.5 | 0.224** |
| Greater than 350ft | 14 | 87.3 | |
| **Sanitation observation within 10metres of pipeline** | | | |
| Yes | 21 | 75 | |
| No | 59 | 81.9 | 0.002* |
| **Defecation near the stream** | | | |
| Yes | 128 people | 49.21 | 0.000* |
| No | 352 people | 53.69 | |

Key: * - significant; ** - not significant.

NB: P-value =<0.05 significant, >0.05 not significant

**Table 12:** Chi square analysis showing effects of antibiotics to sensitivity resistance of some salmonella spp.

| Salmonella | | | | Total |
|---|---|---|---|---|
| Antibiotics | Sensitive | | Resistance | |
| Streptomycin | Count | 17 | 0 | 17 |
| Sensitive | Expected count | 3.8 | 13.2 | 12.0 |
| Streptomycin | Count | 37 | 0 | 37 |
| (Resist) | Expected count | 8.2 | 28.8 | 37.0 |
| Septrin(S) | Count | 12 | 0 | 12 |
| | Expected count | 2.7 | 9.3 | 12.0 |
| Septrin(R) | Count | 42 | 0 | 42 |
| | Expected count | 9.3 | 32.1 | 42.0 |
| Tarivid(S) | Count | 0 | 19 | 19 |
| | Expected count | 4.2 | 14.8 | 19.0 |
| Tarivid(R) | Count | 0 | 35 | 35 |
| | Expected count | 7.8 | 27.2 | 35.0 |
| Pef (S) | Count | 0 | 6 | 6 |
| | Expected count | 1.3 | 4.7 | 6.0 |
| Pef(R) | Count | 0 | 32 | 32 |
| | Expected count | 7.1 | 24.9 | 32.0 |
| Aug (S) | Count | 0 | 22 | 22 |
| | Expected count | 4.9 | 17.1 | 22.0 |
| Aug (R) | Count | 0 | 32 | 32 |
| | Expected count | 7.1 | 24.9 | 32.0 |
| Cipro (S) | Count | 0 | 24 | 24 |
| | Expected count | 5.3 | 18.7 | 24.0 |
| Cipro (R) | Count | 0 | 30 | 30 |
| | Expected count | 6.7 | 23.3 | 30.0 |
| Gent(S) | Count | 0 | 10 | 10 |
| | Expected count | 2.2 | 7.8 | 10.0 |
| Gent(R) | Count | 0 | 44 | 44 |
| | Expected count | 9.8 | 34.2 | 44.0 |
| Ampici (S) | Count | 0 | 3 | 3 |
| | Expected count | 7 | 2.3 | 3.0 |
| Ampicillin (R) | Count | 0 | 51 | 51 |
| | Expected count | 11.3 | 39.7 | 51.0 |
| Nalidic(S) | Count | 0 | 9 | 9 |
| | Expected count | 2.0 | 7.0 | 9.0 |



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|---|---|---|---|---|
| Nalidic(R) | Count | 0 | 45 | 45 |
| | Expected count | 10.0 | 35.0 | 45.0 |
| Total | Count | 108 | 378 | 486 |
| | Expected count | 108.0 | 378.0 | 486.0 |

X2 = 486, DF = 17, P = 0.000 (P<0.5, significant)

## Discussion

The isolation of *Salmonellaspp* from water sample means that the direct consumption of such water without treatment May be very risky. The genus is known to cause *Salmonellosis.* The typhi and paratyphi species causes typhoid fever which can be spread through contaminated water.

This study, as expected shows that there is high bacteria load in the surface river water sample. These findings are in agreement with those reported by Doughari et al. [16] in Tiete River in Brazil and Adednego et al. [17] in Nairobi respectively. This study reveals that contamination of this surface water is by humans and other animal sources like bathing, farming and washing.

Surface waters from Oraukwu and Alor recorded high prevalence rate of *Salmonella species*. This is not surprising because some members of this community carryout a lot of farming activities near the surface water; some of them defecate along the farm land since their house is far from the farm, moreover, they all use bare hand to pack animal manures used for fertilizer on the farm land.

There is tendency that the surrounding flood from these farm lands may be carried into the stream. Also, this work also reveal that some of the spring waters from St. John Alor and Oraukwu (Nwaocha) recorded no bacteria growth at different spots collected. This may be attributed to the suggestion that spring water has natural filter that filters the water while a lot of indicator organism and *Salmonella* were recorded on the same source collected at different spots. This is attributed to the fact that contamination may be as a result of human activities like bathing, farming, washing etc.

From this present study, it shows that boreholes can also be contaminated through flood water which forms after rainfall, depending on the depth of the boreholes. Deeper boreholes contain little or no micro organisms since they are usually removed by extensive filtration as water percolates through the soil [18] as shown by the Nnobi borehole constructed by the government with depth 350ft when compared to borehole constructed by individuals in Alor, Oraukwu and Abatete who have little or no knowledge about importance and role of depth in sanitation of water.

Contamination could also be through broken underground pipes under this condition, the surrounding flood water flows into the pipe through the cracks [19]. Orji et al. [20] and several others have reported similar findings and recommended that household boreholes should be sited far away from soak-away pits and latrines.

*In vitro* disc susceptibility studies of the different isolates of *Salmonella* species obtained from the water sample showed that 9 drugs were susceptible isolates at different level. No isolate was sensitive to penicillin (30mg) 0(0%).Ciprofloxacin (40mg) was susceptible to 24(44.4%) of the isolated *Salmonella* species, it was the drug most sensitive to the different species of *Salmonella.*

The bacteriological analytical method recovered more of *Salmonella* species than membrane filtration method. In membrane filtration method, pressure from the filtration may have stressed the organisms, some of them are being lost but Bacteriological Analytical Method require only dilution method and the organisms are not stressed, this may contribute to high recovery rate of *Salmonella* species with the methodology. This also in similar with the work done by Umeh [21] on isolation of *Salmonella* species from powdered infant formular sold in Nnewi market.

## Conclusion

The result of this study revealed that the water sources in these communities are highly polluted and is not safe for use by the communities for drinking or other domestic needs without prior treatment. There is need to monitor regularly and mitigate the effects of community behavior on surface waters. The results also indicate that some of these *Salmonella spp.* are resistant to some of the common antibiotics which if neglected becomes a very serious public health problem.

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