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Occurrence of *Salmonella* in Surface and Borehole Waters from Four Coastal Communities in Bayelsa State

*Ohimain, E.I[1], Zige, D.V.[1] and Sridhar, M. K. C[2],
[1]Public Health and Medical Microbiology Research Unit Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria
[2]Department of Environmental Health Sciences, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: *In spite of Millennium Development Goals coming to a close, safe water and sanitation pose universal challenges for public health as water-borne diseases dominate the leading causes of morbidity and mortality. In developing countries, about 2.2 million people die every year due to basic hygiene-related diseases, and typhoid is on the increase. The major route of transmission is contaminated water. Salmonella of human origin indicates pollution from human wastes. Four river water and three borehole water samples were collected in four rural coastal communities of Bayelsa state, Nigeria and analysed for total coliforms and S. Typhi. Samples were filtered by a membrane filter of pore size 0.45µm (Milipore) and enriched on Selenite F broth and incubated for 24h followed by plating on Salmonella-Shigella Agar. A total of 10 isolates were identified as Salmonella (S. Typhi, 70.85% and non-Typhi, 29.15%) by conventional biochemical test. In addition, the samples were analysed using Membrane filter techniques for coliforms count, thermotolerant coliforms count, and heterotrophic organisms at different incubation temperatures for mammalian and environmental source of contamination. Salmonella was detected in all water samples with the exception of borehole water samples from Bumoundi-Ekpetiama however Antibiotic susceptibility pattern of isolated Salmonella Typhi showed that isolates were 100% susceptible to Tetracyclin, Chloramphenical, Ciprofloxacin and Nalidixic acid while 100% resistant was observed for amoxicillin and for the Non-Typhi Salmonella. Chloramphenical, Cotrimoxazole, Ciprofloxacin, Cephalexin, Nalidixic acid, and Tetracyclin were 100% susceptible, whereas amoxicillin was 60% resistant, on the other hand Ceftizoxime was 20% resistant as well. The microbiological load of Salmonella from surface waters is a concern for the rural dwellers as they use the river for defecation, disposal of wastes and use for their drinking and other needs. The study recommends appropriate intervention to check Salmonellosis and other water-borne infections.*
Key-words: *Surface water, River water, Water-Borne Disease, Coliforms, Salmonellae, Water Pollution, Typhoid fever, Borehole water.*

I. Introduction

*Salmonella*e are Gram-negative, motile bacteria belonging to the family Enterobacteriaceae. They are generally non-capsulate and non-spore forming with the exception of *Salmonella* Typhi (7). *Salmonella* is ubiquitous in animal populations and human illness is usually linked to foods of animal origin (12). Salmonella infections may be divided into five categories: gastroenteritis, enteric fever, bacteraemia, localized infections, and the chronic carrier state (22). The most serious localized manifestations outside the gastrointestinal tract include endovascular lesions, osteomyelitis, and meningitis. Vascular infections due to Salmonella can involve the thoracic and abdominal aorta, as well as coronary arteries, peripheral arteries, or vascular grafts and prosthetic valves (22). In regard to enteric illness, *Salmonella* spp. can be divided into two groups: the typhoidal serovars (*Salmonella* Typhi and *S.* ParaTyphi) and the non-typhoidal serovars, *Salmonella* diseases afflicted mankind since human populations grew large enough to contaminate their water and food supplies. Enteric fever (typhoid or paratyphoid fever) is a systemic infection caused by several *Salmonella enterica* serotypes including *S.* Typhi and *S.* ParaTyphi A, B, or C, with human beings as the only known natural hosts and reservoir of infection (13). Enteric fevers have continued to pose serious threat to public health especially in economically poor countries where the level of hygiene is below standards and sanitary conditions are poor (11). Estimates for the year 2000 suggest that there are approximately 21.5 million infections and 200,000 deaths from typhoid fever globally each year (9; 6), Attributable deaths annually is predominantly among children under the age of five years (8). In Africa, about 4.36 million cases occur out of an estimated population of 427 million and it is often encountered in tropical countries including Nigeria where they constitute serious source of morbidities and mortalities (14). *Salmonella* are widely distributed in the environment, but some species or serovars show host specificity. The pathogens typically gain entry into water systems through faecal contamination. Water-borne *Salmonella* outbreaks have devastating public health implications. The World Health Organization (WHO) estimated that about 80% of ill-health in developing countries especially are water related (7).

Inadequate potable water supplies are a serious socio-economic problem in several riverine communities in Nigeria and this has caused the inhabitants to resort to polluted river water for domestic water supplies. Polluted and untreated water supplies are responsible for water-borne infections such as enteric fevers. Morbidity associated with illnesses due to *Salmonella* continues to be on the increase and, in some cases, resulting in mortality (16). To date, it has been attributed to consumption of contaminated food products and water (18). The issue of contaminations of fresh water sources available are on the increase especially in local riverine communities as pollution is not just of faecal origin, but also noxious substances containing organic wastes that promote the growth of pathogenic bacteria, (2).

In the developing world, an estimated 10 million children under-five die of diarrhea related diseases of these deaths, WHO estimates that 16.5 percent, or at least 1.65 million, were caused by contaminated water (25). Deaths caused by nondiarrheal infections like typhoid fever are also related to contaminated water (9). (20) found that 10.7 million infections and 5.4 million illnesses/year occur in populations served by community groundwater systems; 2.2 million infections and 1.1 million illnesses/year occur in noncommunity groundwater systems; and 26.0 million infections and 13.0 million illnesses/year are reported in municipal surface water systems as water can be a carrier of many water-borne diseases, such as typhoid, cholera, hepatitis, dysentery and other diarrhoea-related diseases. Shortage of clean drinking water has been an issue faced with most coastal and riverine communities in Nigeria. This situation prevailed in many developed countries in the past some three centuries ago, until measures for sanitary disposal and supply of potable water were put in place. There is enough evidence to show that the prevalence of typhoid in any community is an index of communal hygiene and effectiveness of sanitary disposal (4). The poor sanitation and unhygienic practices are evident in both urban and rural areas of Nigeria as shown in Figure 1.

Figure 1; Unhygenic practices of local dwellers in Bayelsa State in Nigeria.

Pictures :(a) fetching water close to toilet houses (b and c) depositing house hold waste from kitchen pots around a toilet house (d) waste on river water (e) deposited waste on river banks (f) toilet house

Salmonellosis is considered one of the most serious infectious disease threats to public health globally, with particular concern over the rapid and widespread emergence of resistance to multiple antibiotics (3; 26). This paper reports the dominant isolates, and varieties found in a common water sources of four communities in Bayelsa State in South-South Nigeria and describes possible risks posed by the exposed communities.

II. Materials and Methods

Study Area

This study was carried out in three Local Government Areas in Bayelsa state. Facilities at the Federal Medical Centre, Yenagoa and Niger Delta University, Wilberforce Island were used (Table 1 and Figure 2).

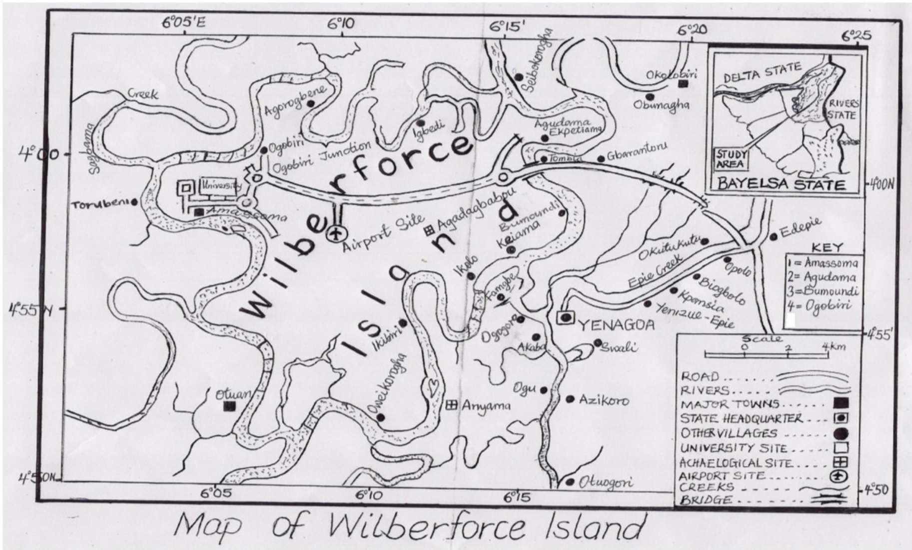

Figure 2; Map showing the Coastal Communities of the three Local Government Area

Table 1. Collection sites of River Water Samples

Accession	Local Government Area	Latitude	Longitude
Amassoma	Sourthern-Ijaw	4^0 58. 482'	6^0 6. 3144'
Ogobiri	Sagbama	$4^0$59.9676'	6^0 7. 2462'
Agudama-Ekpetiama	Yenagoa	5°0.5045'	6°15.705'
Bumoundi-Ekpetiama	Yenagoa	4^o58.7652'	6°16.1927'

The Communities under Study

Amassoma

Amassoma is located about 40 km to the South of Yenagoa the State capital. It is bounded to the North by River Nun, West by Otuan and Wilberforce Island, East by Toru-Ebeni and the South by Ogobiri. The population is heterogeneous and second most populous place in the state, consisting of the native Ogboins, students, petty traders and businessmen from other parts of the State and country. It hosts institutions of learning and Health. The community also records total absence of refuse disposal, pipe borne water, electricity, social and recreational facilities with few commercial boreholes in the community.

Ogobiri

Ogobiri is a neighbouring community to Amassoma located in Wilberforce Island with a homogenous population of about 1200 people. The community also host institution of learning and dilapidated health care centre, the scenario for waste disposal, electricity and social recreational facilities are the same with Amassoma. For this community there are no boreholes water and the common source of water for domestic activities is the surface river water unlike Amassoma.

Agudama-Ekpetiama

This community is bounded to the south by river nun and Tombia, the north by Egbedi, west by Bumoundi and east by Polaku, located in Wilberforce Island with a homogeneous population of about 1200 people and 107 houses consisting mostly of the Agadas, who mostly engage in fishing and farming. This community hosts institutions of learning and Health. The community records the presence of electricity and borehole with no proper waste disposal and recreational activities.

Bumoundi-Ekpetiama

This community also located in Wilberforce island with a homogeneous population of about 1200 people and 96 houses consisting mostly of the Agada, who mostly engage in fishing and farming. This community hosts institutions of learning and dilapidated health care centre. The community also records presence of pipe borne water, boreholes and total absence of refuse disposal, electricity, social and recreational facilities.

Water Sampling and Analysis

A total of four surface river water samples were collected from four communities. Water samples were collected about 24.4m away from the shore and three borehole waters that are major sources of public supply.

Surface river water Samples were collected aseptically in 1000 ml bottles and borehole water samples were collected in 1000 ml bottles containing 2 ml freshly prepared 0.4 N sodium thiosulphate for microbiological analysis. The samples were preserved in an icebox during transportation, brought to the laboratory and analysed within 6h of collection (7).

Bacterial Isolation and Identification
Total and Faecal Coliform Count

Total coliforms were enumerated by the membrane filtration method. Water samples were filtered aseptically through a millipore membrane filter of pore size 0.45 µm. The membrane filter was transferred and placed aseptically on MacConkey agar and the plate was incubated at 37ºC (Total coliforms) for 24 h and at 44.5ºC (*E coli*) for 24 h. Water samples were analysed in duplicate and enumeration for total colony forming units (CFU) were based on mean values (5 and 19).

Heterotrophic plate count

Total heterotrophic plate counts were carried out by the membrane filtration method as described above but using nutrient agar plates Incubation was carried out at 37°C for 24 h to enable the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources. Enumeration was based on average of each sample analysed (15).

Isolation and Identification of *Salmonella*

The water samples (100 ml) were aseptically filtered through a Millipore membrane filter (0.45 µm). The membrane filters were enriched in 10 ml sterile Selenite F broth for 24 h at 37ºC. A loopful of the suspension from the positive tubes were streaked on the Salmonella-Shigella agar plates and incubated at 37ºC for 24-48 h. All the pale and black centred colonies were considered as presumptive for *Salmonella,* hence were purified on Nutrient agar. The pure cultures were identified by the conventional biochemical methods (21; 12).

Antibiotic Susceptibility Test of *Salmonella* Isolates

The in-vitro sensitivity test for the salmonellae isolates identified using biochemical reactions, were carried out. The Sensitivity of the pure culture of bacteria isolates to different antibiotics was determined using the Kirby- Bauer disc diffusion technique and interpreted based on the guidelines of the Clinical and Laboratory Standards (23). The antibiotics (Abtek biological Ltd) discs used contained the following antibacterial agents: Augmentin (30ug), Ofloxacin (5ug), Gentamicin (10ug), nalidixic (30ug), Nitrofuratoin (200ug), Cotrimoxazole (25ug), Amoxillin (25ug), Tetracycline (25ug). Mueller-Hinton (MH) agar plates were swabbed with cells from the bacteria stock solution, already adjusted to the 0.5 McFarland's turbidity standard. The discs were thereafter, carefully placed on the agar and incubated at 37^{o}C for 24h. Interpretation of the strains as sensitive or resistance were based on zones of inhibition according to current NCCLS standards in accordance with WHO requirements (24).

III. Results

Total coliforms were detected in 100% of the samples and *E coli* in 23.3% of the samples. Higher percentage (50%) of coliform was isolated from Amassoma (Table 2). The heterotrophic organisms of mammalian origin are of less percentage to those of environmental origin (Table 2). All surface river water samples analysed were positive for *Salmonella* (Table 2) identified using conventional biochemical methods (Table 3); of which 75 % was *S*. Typhi while 25% were non-Typhi *Salmonellae*. Borehole water samples were 66.7% positive for *S*. Typhi and 33.3% positive for non-Typhi *Salmonellae.*

Antibiotic susceptibility pattern of isolated *Salmonella* Typhi showed that isolates were 100% susceptible to Tetracyclin, Chloramphenical, Ciprofloxacin and Nalidixic acid while Cephalexin has 90% susceptibility, Ceftizoxime 80% susceptibility and Cotrimoxazole 60% susceptible. 100%, resistant was observed in amoxicillin (Table 4). For Non-Typhi *Salmonellae,* Chloramphenical, Cotrimoxazole, Ciprofloxacin, Cephalexin, Nalidixic acid, and Tetracyclin were 100% susceptible, while amoxicillin was 60% resistant and to Ceftizoxime 20 % resistant (Table 5).

Table 2; Microbial count of surface River water and drinkable water

Total coliform	Communities/Location							
	Amassoma		Ogobiri		Agudama-Ekpetiama		Bumoundi-Ekpetiama	
	SRW	BHW	SRW	BHW	SRW	BHW	SRW	BHW
CFU / 100 mL	152	16	123	No borehole	54	14	48	3
Thermotolerant (*E coli*) **(CFU/ 100 mL)**	34	8	17	No borehole	2	1	12	No colonies
Heterotrophic (37°c) **(CFU / 100 mL)**	88	23	66	No borehole	11	11	22	No colonies
Heterotrophic (22°c) **(CFU / 100 mL)**	44	43	48	No borehole	42	21	11	7
Salmonellae occurrence	+	+	+	No borehole	+	+	+	No colonies

Key
+ Colonies
SRW-Surface River Water
BHW-Borehole Water

Table 3; Biochemical Differentiation of Isolated *Salmonella* Group

Communities								
	Amassoma		Ogobiri		Agudama-Ekpetiama		Bumoundi-Ekpetiama	
*S*almonellae occurrence	SRW	BHW	SRW	BHW	SRW	BHW	SRW	BHW
S. Typhi	+	+	+	No borehole	+	+	_	_
S. Paratyphi A/B	_	+	+	No borehole	_	_	_	_
Other *S*almonellae	+	+	_	No borehole	+	_	+	_

Key
+ Colony Present
— Colony Absent
SRW-Surface River Water
BHW-Borehole Water

Table 4; Antibiotic sensitivity pattern of *S.* Typhi isolated

S/N	Antibiotics	Resistant (%)	Intermediate (%)	Sensitivity (%)
1	Amoxycillin	100	0	0
2	Chloramphenical	0	0	100
3	Cotrimoxazole	0	40	60
4	Ciprofloxacin	0	0	100
5	Cephalexin	10	0	90
6	Nalidixic acid	0	0	100
7	Ceftizoxime	20	0	80
8	Tetracyclin	0	0	100

Table 5; Antibiotic sensitivity pattern of Non-*S.* Typhi isolated

S/N	Antibiotics	Resistant (%)	Intermediate (%)	Sensitivity (%)
1	Amoxycillin	60	40	0
2	Chloramphenical	0	0	100
3	Cotrimoxazole	0	0	100
4	Ciprofloxacin	0	0	100
5	Cephalexin	0	0	100
6	Nalidixic acid	0	0	100
7	Ceftizoxime	20	40	40
8	Tetracyclin	0	0	100

IV. Discussion

Water has been a good source of transmission of pathogens and particularly those of faecal origin. It is therefore imperative that the safety of surface and ground water intended for drinking should be protected. Though surface waters are more prone to contamination of all kinds deaths caused by nondiarrheal infections like typhoid fever are in many cases related to contaminated water whether it be surface or underground (9). This study, as expected shows that there is a high bacterial load in the surface river water sample. These findings are in agreement with those reported by (10), in Gudu stream Abuja, (17) in Tietě River in Brazil and (1) in Athi River in Nairobi respectively. This study also reveals that Contamination of these surface river water is by humans and other animal sources. However, the results for the borehole water shows that the water had less population of bacteria compared to the surface water but still do not meet the WHO standards for potable water quality. Colonies observed in the borehole water were basically from coliforms that are not thermotolerant and heterotrophs that are not of mammalian origin which shows that there may be leakages of pipes or open storage tanks. There is therefore need for quick response to avoid further contamination. The surface river water sample containing high bacteria load is as a result of human activities such as bathing, washing, and discharge of toilet contents. These communities build toilets on river and disposed the wastes directly into the water body. The occurrence of *Salmonella* of clinical relevance in any water samples is of a potential risk, because it is not part of the normal flora of the intestine. And if not responded early, there could be outbreaks of typhoid and other faeco-oral infections as it is happening currently in Nigerian communities.

V. Conclusion/Recommendations

The result of this study revealed that the surface waters in the coastal communities in Bayelsa State 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 behaviour. The results also indicated that some of these *Salmonella* spp are resistant to some of the common antibiotics which if neglected become a very serious public health problem.

In response to this, government, individuals and relevant agencies should come to the aid of these rural dwellers by providing the basic sanitary necessities such as safe water supply and hygienic toilets. Also the common practice of building of flush toilets on water bodies or connecting the drainage to the river directly should be prevented through legislation and imposing penalties. There should be high level of awareness campaign and health education on human waste management and other hygiene behaviours.

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