



Original Research Article

## A Community Based Screening of Asymptomatic Typhoid Carriers in Wilberforce Island, Bayelsa State, Nigeria

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### ABSTRACT

Infectious diseases are of public health concern throughout the world, so constant vigilance is required to prevent the reappearance of diseases and conditions thought to have been conquered. Typhoid fever is a bacterial disease, caused by *Salmonella Typhi*. It is transmitted through the ingestion of food or liquids contaminated by the faeces or urine of infected people. Typhoid fever is still a disease of burden in rural area especially in coastal communities including Wilberforce Island, a town known for its tertiary Institute and a Teaching Hospital serving the communities around. Routine monitoring of the sources of infection and the spread will help in reducing the menace. Stool samples (360) from asymptomatic subjects were screened for *Salmonella Typhi* using recommended laboratory procedures and identified using standard biochemical tests. , Samples were further confirmed and characterised using *Salmonella* sero-group kit. The results of the characterization and identification of the isolates revealed that *Salmonella Typhi* had the highest frequency of occurrence with 57 (48.7%) isolates followed by *other Salmonella serogroup* 24 (20.5%), *Salmonella paratyphi A* (11.1%), *Salmonella paratyphi C* 12 (10.3%), and *Salmonella paratyphi B* 11 (9.4%).The distribution of *S. Typhi* from stool samples of asymptomatic carriers on the basis of sex out of the 57 positive isolate shows that the frequency is higher in females (61.4%) than males (38.6%) and the frequency on the basis of age, frequency is highest among age 10 and below (33.3%). While the least frequency is seen among age range of 11-25 (8.8%), antibiotic susceptibility pattern of isolated *Salmonella* showed that isolates were multi drug resistant strains, which may be as a result of indiscriminate use of the few available antibiotics over the counter and/or inaccurate or inconclusive diagnosis and incomplete antibiotic use resulting in the development and spread of resistant strains of *S. Typhi*. This study however recommends, surveillance programmes to monitor the spread and antimicrobial resistance patterns in the island and other parts of the state and the country at large.

**Key words:** *Salmonella* carriers, Antimicrobial susceptibility, Multidrug resistance, Frequency, Wilberforce Island.

## INTRODUCTION

For quite a lot of contagious diseases, there are persons who pass on their illness but do not exhibit any symptoms. These individuals are called "carriers" and they play a substantial role in spreading of disease in the communities. There are two types of carriers. Inherent carriers who carry the illness on genes that not expressed referred to as "recessive" and can only pass on their disease to their children making it noncontagious. This study aimed at asymptomatic carriers of *Salmonella Typhi* in rural coastal areas of Bayelsa State in southern Nigeria. These respondents involved in the study are asymptomatic and are likely unaware of their health conditions, and therefore are more likely to infect others. An infectious disease that produces long-term asymptomatic carriers is the Typhoid fever caused by *Salmonella Typhi*. Typhoid is one of the most wide spread of all bacterial diseases in the world. The main source of typhoid is asymptomatic carriers. An individual can asymptotically carry the typhoid germ for days to years without showing any symptoms of typhoid fever making it a major public health problem in the worlds. In such carriers, the typhoid bacillus continues to multiply in the gall bladder, it reaches the intestine through the bile duct.<sup>[21]</sup> Typhoid fever reached public notoriety at the beginning of the 20th century with the cases of Typhoid Mary in the US and Mr. N the milker" in England. These individuals infected hundreds of people over the decades while they worked in the food production industry and private homes. Even today there are approximately 21.5 million infections and 200,000 deaths from typhoid fever globally each year.<sup>[4,6]</sup> In Africa, about 4.36million 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.<sup>[10]</sup> Asymptomatic carriers are believed to play an essential role in the evolution and global transmission of *Salmonella Typhi*, and their presence are a great setback to eradication of Typhoid fever using routine treatment and vaccination.<sup>[15]</sup>

Nigeria like many other tropical developing countries has been described as endemic zone for typhoid fever.<sup>[25]</sup> It is thus considered one of the most serious infectious disease threats to public health globally. However, there has been a growing concern about the prevalence of Multidrug Resistant (MDR) *Salmonella Typhi* in developing nations, Nigeria inclusive.<sup>[11]</sup> Most of the MDR *Salmonella Typhi* indicates plasmid mediation to be of importance. Resistance to the routinely prescribed antibiotics is usually plasmid-mediated and has been reported widespread from most parts of the world.<sup>[8]</sup> The disease is a cause of concern in coastal area of Nigeria due to poor sanitary conditions and lack of inadequate potable water especially among rural dwellers.

The drug of choice for treating typhoid fever in areas where, Multidrug-Resistant (MDR) *Salmonella Typhi* strains are prevalent is Ciprofloxacin. However in recent years there are cases of Ciprofloxacin resistant strains reported among *Salmonella Typhi*, leading many clinicians to question the efficacy of this drug. Decreased susceptibility to ciprofloxacin has been reported from Vietnam, Tajikistan, and Nigeria. The screening of asymptomatic carriers who can act as a source of infection and also finding out the emergence of drug resistance by antimicrobial susceptibility test is an integral part in the monitoring, control and eradication of typhoid fever in endemic zones including Nigeria.

## MATERIALS AND METHODS

### Study Area

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

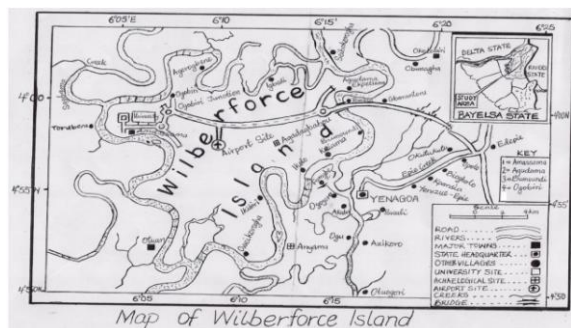


Figure 1. Map showing Wilberforce Island of Bayelsa state Nigeria.

Wilberforce Island is located about 40 km to the South of Yenagoa the State capital. It is bounded by River Nun, the island comprises of about ten (10) communities and few hamlets, the population is heterogeneous and second most populous place in the state, consisting mostly of the native Ijaws, students from neighbouring areas, petty traders and businessmen from various parts of the State and others. It hosts higher institutions of learning and a Teaching Hospital. About 90% of the communities also lacks sanitary facilities, refuse disposal facilities, pipe borne water, electricity, social and recreational facilities and only a few enjoy boreholes and many buy water from vendors.

### Collection of Samples

#### Stool samples

A total of 380 stool samples (equal proportion from both male and female) were collected from individuals from from 1-65 years within October 2012 to July 2013 in Wilberforce Island. Samples were collected following routine laboratory procedures in universal bottles and a loopful of sample was inoculated into selenite F broth and

transported in a cold ice box to the Department of Medical Microbiology, Federal Medical Centre-Yenagoa, Bayelsa State for screening tests. Samples in selenite F broth were incubated for about 18-20hrs for the recovery of isolates after which were inoculated into *Salmonella-Shigella* agar prepared according to manufactures instructions using the streak plate method and incubated for 24hrs to observe black centred colonies.

### Biochemical Identification

All presumptive colonies obtained from the culture plates were screened by using the following media/tests: the purpose of this test is to differentiate *Salmonella* Typhi and *Salmonella* Paratyphi (A,B,C) based on hydrogen sulphide ( $H_2S$ ) production, gas production, motility test, indole test, urease test and sugar fermentation. [26]

### Kligler's Iron Agar

These media were prepared following manufacturers instruction; a sterilised needle was used to pick from the suspected colony that was subcultured on *Salmonella-Shigella* agar and stab through the centre of the agar to the bottom of the tube and also the surface of the slant was streaked. This double inoculation allows growth of unknown organisms on the aerobic surface of the slant and in the largely anaerobic butt of the tube and incubated for 24 hrs before interpretation of the results. The production of acid turns the agar yellow. For the slant this means lactose fermentation and for the butt this means glucose fermentation. If an organism forms  $H_2S$ , the lower portion of the tube will turn black, due to formation of iron sulphide it will be read as positive, if an organism forms gas from glucose or lactose the agar in the butt will show bubbles or cracks. Interpretation of result was as described by. [5]

### **Motility, Indole, Urease Test**

Same wire loop used for the Kiger Iron Agar was used to stab to the centre of the medium prepared using 2.3g of peptone water in 140ml, after which 3 drops of phenol red and 1.5g of agar-agar was added and autoclaved at 121<sup>o</sup>c for 15min. Prior to inoculation, the medium was brought to room temperature and dispensed to tubes and allowed to solidify, then the tubes were incubated aerobically at 35<sup>o</sup>C for 18-24 hrs. Observation for motility and urea, after reading the above reactions, 2 drops of Kovacs reagent was added and observed for indole production.

### **Serological Confirmation of Salmonella Spp**

Biochemically identified isolates of salmonella were grouped using salmonella sero-group kit from Statens Serum Institute ranging from A-G and Vi antisera, the slide agglutination was carried out as described by the manufacturer, the test was done to narrow the search for Vi positive *Salmonella* strains and as indicative of *S. Typhi* as well as other serogroups and interpreted as described by the manufacturer.

### **Antimicrobial Susceptibility Testing**

The in-vitro sensitivity test for the isolates identified using serological confirmation, were carried out, 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.<sup>[24]</sup> The antibiotics (Abtek biological Ltd) Discs used in the study contained the following antibacterial agents: Ofloxacin (5µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ceftazidime (30µg), Nitrofuratoin (300µg), Augmentin (30µg), Cefixime (5µg) and Cefuroxime (30µg). Oxoid sensitest agar plates were swabbed with cells from the

bacteria stock solution, preadjusted to the 0.5 McFarland's turbidity standard. The discs were thereafter, carefully layered on the agar and incubated at 37<sup>o</sup>C for 24Hrs. Interpretation of the strains as sensitive or resistance were based on zones of inhibition according to current NCCLS standards in accordance with WHO requirements.<sup>[23]</sup>

## **RESULTS**

The study screened 360 stool samples of asymptomatic subjects, out of which 117(32.5%) were positive for *Salmonella* infection indicating there is carriership of this bacilli among asymptomatic individuals in the study area. The results of the characterization and identification of the isolates revealed that *Salmonella Typhi* had the highest frequency of occurrence with 57 (48.7%) isolates followed by *other Salmonella* serogroup 24 (20.5%), *Salmonella Paratyphi A* (11.1%), *Salmonella Paratyphi C* 12 (10.3), and *Salmonella Paratyphi B* 11 (9.4%), in a descending order as shown in Table 1.

**Table 1: Distribution of Salmonella isolates.**

Organism	No of positive (117)	Frequency (%)	Frequency (%) N=360
<i>S. Typhi</i>	57	48.7	15.9%
<i>S. Paratyphi A</i>	13	11.1	3.6%
<i>S. paratyphi B</i>	11	9.4	3.1%
<i>S. paratyphi C</i>	12	10.3	3.3%
Other serogroup	24	20.5	6.7%

The distribution of *S. Typhi* from stool samples of asymptomatic carriers in the study area on the basis of sex out of the 57 positive isolate shows that the frequency is higher in females (61.4%) than males (38.6%) and the frequency on the bases of age, frequency is highest among age 10 and below (33.3%). While the least frequency is seen among age range of 11-25 (8.8%) (Table 2).

**Table 2: Distribution of S. Typhi of carriers on the bases of sex and age.**

Age Group	Number Examined	Sex		Number of positive by sex (S. Typhi)		Sum of positives	Total Freq (%)	Age Freq rate (%)	
		Male	Female	Male	Female			Male	Female
≤10	90	45	45	12	7	19	33.3	63.2	36.8
11-25	90	45	45	1	4	5	8.8	20	80
25-35	90	45	45	4	11	15	26.3	26.7	73.3
35≥	90	45	45	5	13	18	31.6	27.8	72.2
Total	360	180	180	22	35	57	100	34.4	65.6
Frequency rate (%)				38.6	61.4	100			

Key: Freq= Frequency

The *in vitro* susceptibility profile of *Salmonella* Typhi on the following antibiotics, Ofloxacin (5µg) is 60%, and Ciprofloxacin (5µg) 90% while Gentamicin (10µg), Ceftazidime (30µg), Nitrofurantoin (300µg), Augmentin (30µg), Cefixime (5µg) and Cefuroxime (30µg) is 0% which are drugs used routinely for the treatment of gastroenteritis in the study area. Complete susceptibility was observed for other

serogroup of *Salmonella* isolate to ciprofloxacin (5µg), following *Salmonella* Typhi and *Salmonella* paratyphi (A,C,B) (Table 3) however the result of the susceptibility pattern for *Salmonella paratyphi* (A,B,C) isolated from asymptomatic carriers stool culture are also shown in (Table 3) revealing complete resistance of Ceftazidime (30µg) and Cefuroxime 30ug.

**Table 3: Percentage of antibiotic susceptibility profile of salmonellae isolated.**

Organism	No of isolates	Ofloxacin (5µg)	Augmentin (30µg)	Cefixime (5µg)	Ciprofloxacin (5µg)	Ceftazidime (30µg)	Cefuroxime (30µg)	Nitrofurantoin (300µg)	Gentamicin (10µg)
<i>Salmonella</i> Typhi	57	60	R	40	90	R	R	R	R
<i>Salmonella</i> Paratyphi A	13	53.8	R	50	90	R	R	7.9	23.1
<i>Salmonella</i> Paratyphi B	11	100	81.8	R	36.4	R	R	9.1	R
<i>Salmonella</i> Paratyphi C	12	8.3	R	R	58	R	R	R	16.7
Other <i>Salmonella</i> Serogroup	24	66.7	R	45.8	100	R	R	R	33

R=Resistance

## DISCUSSION

Typhoid fever has been reportedly endemic in Nigeria and has continued to be on the increase all year round.<sup>[7,14,19]</sup> and carriership is attributed to consumption of contaminated food products and water.<sup>[13]</sup> In this study, all the 360 stool samples collected from apparently healthy individuals from Wilberforce Island, Bayelsa State, Nigeria, the prevalence rate of healthy carriers for *S. Typhi* is 15.9% in the communities under study. This findings

however are similar with those of <sup>[21,3]</sup> Transmission of typhoid fever is chiefly through the asymptomatic carriers those who work as the food handlers in hotels and restaurants.<sup>[17]</sup> Many of them become asymptomatic carriers after an acute typhoid episode. These infection already established in their gall bladder after the symptoms disappeared, serve as a reservoir for the spreading of the typhoid fever.<sup>[12,16,20,9]</sup> The results also revealed a high prevalence of typhoid fever (33.3%) among children



within the age group ( $\leq 10$ ) years which is also similar to the findings of earlier report of 43.9% and 44.4% prevalence rate in Cebu City and Minna, Nigeria.<sup>[22,1]</sup> It is rational that children are the most vulnerable group in the environments where inadequate water supply and poor environmental hygiene are problems because of their high level of ignorance as well as immune system development against the bacilli in endemic regions. This study also reveals *S. Typhi* as the dominant clinical serotype of *Salmonella* group carried by asymptomatic carriers in the study area, thus agreeing with the laboratory surveillance record on typhoid fever in study area.

Antibiograms of *Salmonella* isolated revealed resistance to preferred drugs. The susceptibility pattern investigated in the study shows the emergence of drug resistant strains to multiple antibiotics. Thus, the MDR *S. Typhi* is reported in this study and in agreement with findings of various reports from tropical countries as well as Nigeria has shown.<sup>[2,18,8]</sup> The resistance of *S. Typhi* (10%) to ciprofloxacin, however the emergence of Ofloxacin (5 $\mu$ g) is 60%, and Ciprofloxacin (5 $\mu$ g) 90% Gentamicin (10 $\mu$ g), Ceftazidime (30 $\mu$ g), Nitrofurantoin (300 $\mu$ g), Augmentin (30 $\mu$ g), Cefixime (5 $\mu$ g) and Cefuroxime (30 $\mu$ g), pose a big problem regarding the treatment of patient with typhoid fever. Alternative drug suggested includes quinolones, fluoroquinolones as they show reduced susceptibility indicated by in vitro resistance.

Antimicrobials agents may be life saving for malnourished and other immunocompromised patients who have typhoid fever. In endemic regions, the identification of risk factors and relevant route of transmission is essential for the development of rational control strategies. Therefore special considerations are needed to encourage patients to procure and consume a complete regime of antibiotics,

proper sanitation, public health education and vaccination are long-term preventive measures that would improve this situation.

## CONCLUSIONS

Favourable environment for the growth of infectious organisms are seen in most rural places in Nigeria, where improper waste disposal and unhygienic sanitary practices are rampant leading to the proliferation of pathogenic organisms. These practices can lead to severe water pollution leading to large-scale outbreaks of disease. Improvement in hygienic conditions would play a significant role in reducing the incidence of *Salmonella enterica serovar Typhi* infection which is a severe illness and may be life-threatening in patients with an underlying disease.

This study has also further drawn attention to the growing concern of the spread of MDR strains of *S. Typhi* in rural areas of Nigeria where there are no restrictions of antibiotic sale and use and high carriage among asymptomatic persons who stand high chances of distributing these bacilli. Therefore necessary interventions that will help to control this threat of antibiotic resistance, provision of potable water, accurate laboratory diagnosis, health education, and detailed epidemiological investigation in the island and other areas in the state should be of paramount concern. This study however recommends, surveillance programs to monitor the spread and antimicrobial resistance patterns in the island and other parts of the state and the entire country.

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