Original Research Article

Prevalence of Non-Typhoidal *Salmonella* species in Food and Stool samples in Port Harcourt, Rivers State, Nigeria

ABSTRACT

Aim: To assess the prevalence of Non-Typhoidal *Salmonella* spp isolated from food and stool samples in Port Harcourt.

Study Design: This was a cross-sectional study with simple randomized sampling.

Methodology: In this study, 210 stool samples and 210 food samples collected from December 2022 to November 2023 were tested for *Salmonella* using standard bacteriological and biochemical tests. The *Salmonella* species were isolated from the samples using *Salmonella*-Shigella agar (SSA), and Bismuth Sulfite Agar (BSA) after pre-enrichment and enrichment methods had been carried out using peptone water and Selenite F Broth after which biochemical tests were carried out for further identification. Data collected was analyzed with Graph pad prism version 8.

Results: The prevalence and distribution of *Salmonella* were presented in frequencies and percentages with all analysis done at a 95% confidence interval and *P*-values less than .05 were considered significant. From chi-square analysis there was a 3.3% prevalence rate in food samples in comparison to stool samples that had a 2.4% prevalence (*P* value = .56). A higher prevalence was also reported in the female subjects (3.7%) compared to the male subjects (1.0%) (*P* value = .19). There was a statistically significant difference in relation to the age groups with the 'above 50' and '0 – 10' age groups having a higher prevalence (11.1% and 10% respectively) compared to other age groups (*P* value = .02). For the food samples, samples in the chicken category had the highest prevalence (8.7%) (*P* value = 0.11).

Conclusion: This study reports a relatively lower prevalence of Non-Typhoidal *Salmonella* species at 2.9% with the age, education and occupation of the subjects being significantly associated (*P* value < .05) with the prevalence of the infection. Health promotion and appropriate surveillance system should be put in place to continually reduce the burden of this disease.

Keywords: Non-Typhoidal, Prevalence, Salmonella, Salmonellosis, Microbiology

1. INTRODUCTION

Foodborne infections cause enormous suffering, affecting 10% of the world's population resulting in 33 million deaths per year [1]. There are several variables that lead to foodborne

infections and illnesses caused by *Salmonella* species. *Salmonella* belongs to the *Enterobacteriaceae* family which is a gram-negative bacterium with rod-like morphology (bacillus). *Salmonella bongori* and *enterica* are the two species of *Salmonella* that are currently recognized. There are more than 2,600 serotypes of *Salmonella* within the six subspecies of *S. enterica* [2][3].

The serovars, or mosaic combinations of surface O and H antigens, are what distinguish *Salmonella* enterica from its around 2600 closely related species. There are two types of *Salmonella* infections: typhoid and non-typhoidal, which are distinguished by their distinct pathogenic characteristics. Non-typhoidal *Salmonella* (NTS) infections typically resolve on their own, whereas typhoidal *Salmonella* infections have the potential to cause fatal systemic infections [2] [4]. Public health and food safety are seriously threatened by the emergence of pathogenic *Salmonella enterica* serovar Typhimurium, particularly when it comes to multiple antibiotic resistance (AR). *Salmonella* spp. Has been implicated as the top foodborne pathogen in a recent study conducted in the USA, with the highest quality-adjusted life-year (QALY) losses and the highest cost of sickness [5].

Salmonellaenterica subsp. enterica, continues to be the primary cause of infectious gastroenteritis. Animal-derived foods, fruits, vegetables, and water are frequently linked to cases [6] [7] [8]. Annually, non-typhoidal *Salmonella* is responsible for around 155,000 deaths and 93 million cases of gastroenteritis worldwide [9]. Some factors that affect the pattern of disease manifestation include serotype, virulence factors, infectious dosage, and host immunity. From the host perspective, the most affected with more severe clinical signs, such as sepsis are children, elderly, and immunocompromised patients [10]. In certain instances, the infection may result in a long-term, asymptomatic condition of carriage in the host [10]. The most common serotypes of *Salmonella* that cause human salmonellosis are *Salmonella* typhimurium and *Salmonella* enteritidis [11] [12]. However, new serotypes that are growing more common have been found to infect humans in the United States and the world, including *Salmonella* Heidelberg, Javiana, Infantis, and Thompson.

The NTS infections are a significant contributor to pediatric disease. According to a study conducted in the United States on children under the age of five who had laboratory-confirmed cases of bacterial enteritis, the most frequently isolated bacterial enteric pathogen was NTS (42%) followed by *Campylobacter* (28%), *Shigella*, *Escherichia coli* O157, *Campylobacter*, and *Yersinia enterocolitica* [13]. The most frequent pathogen in Taiwan linked to hospitalized cases of bacterial enterocolitis in children is NTS [14]. NTS resulted in between 70 and 80 percent of food poisoning cases in China [15]. As a result, NTS infections in children continue to place a heavy strain on healthcare systems and raise serious concerns for global public health [15].

According to a systematic review, 29% of community-acquired bloodstream infections in Africa were caused by *Salmonella enterica*. In certain regions of the continent, NTS was responsible for a significant portion of these infections, accounting for 88% in eastern Africa, 97% in southern Africa, and 87% in western and central Africa, while only 1% in northern Africa [16]. Furthermore, this research revealed that *S. enterica* serovars Typhimurium and Enteritidis, which together accounted for 65.2% and 33.1% of all NTS serotyped isolates, were the two most frequent serovars producing NTS infections [16]. Certain areas of Africa seem to have a higher prevalence of NTS illness than other parts of the world [17]. In Africa, where the disease is strongly linked to HIV infection in adults [16][18][19] as well as malaria and malnutrition in newborns and children [20][21][22], host risk factors seem to be at the forefront of the disease's epidemiology [23][24].

In Nigeria, the burden of NTS was 325,731 cases with a total of 1043 human fatalities, and 37,321 disability-adjusted life years (DALYs), using 2020 as the reference point. The price tag on human infection was $473,982,068. The estimated overall loss in poultry, including the direct value of animal loss, was $456,905,311 [25]. In other to better understand the epidemiology of Salmonellosis in the region and to direct the adequate care and preventive measures for this disease, this study was aimed at investigating the prevalence of non-typhoidal *Salmonella* species in Port Harcourt, Nigeria.

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2. MATERIAL AND METHODS

2.1 Study Area

Samples for this study were collected from different locations in Port Harcourt, Rivers State, in the oil rich Niger Delta, southern Nigeria. Bulk of the stool samples were collected from Rivers State University Teaching Hospital (RSUTH) and University of Port Harcourt Teaching Hospital (UPTH), others were collected from medical laboratories and identified individuals presenting with gastroenteritis in the Port Harcourt metropolis. Food samples were collected from different fast-food outlets, local 'mama put' and street-hawked food in the study area. Port Harcourt is located between latitude 4°49´27" N and longitude 7°2´1 " E. The study area has an estimated population of 3,480,101 with a land area of 369 km^2

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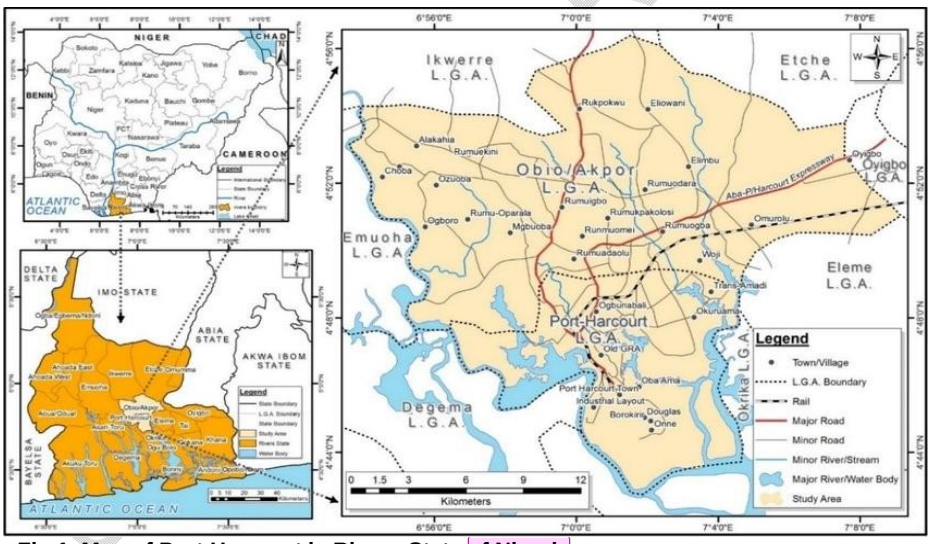

Fig.1. Map of Port Harcourt in Rivers State of Nigeria

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2.2Study Design

The investigations were carried out using a cross-sectional study design with simple randomized sampling technique. The study was undertaken from December 2022 to November 2023

2.3Study Population and Sample Size Determination

The study population comprised patients presenting with gastroenteritis, patients with bacteriologically confirmed *Salmonella* infection and those presenting stool to the laboratory for examination. Different food vendors were sampled in the course of this study. The sample size of 210 for food samples and 210 for stool samples were obtained using a sample size calculator for prevalence studies [26] based on the expected prevalence of *Salmonella* species in stool as reported by [27] which revealed a prevalence of 16.3%.

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2.4Inclusion and Exclusion Criteria

An indication of the signs and symptoms of Salmonellosis such as diarrhea, stomach cramps, fever, nausea, vomiting, chills, headache, blood in stool, etc., as well as willingness to provide informed consent were the basis for inclusion in the study while patients already undergoing antibiotic therapy and those who didn't give consent were excluded from participating in the study. Spoilt and raw food were excluded from this study while street-vended and food sold in restaurants were included in this study.

2.5Data Collection

A questionnaire was developed and given to subjects to obtain sociodemographic and other vital information. The questionnaire comprised of two sections, the first section assessed sociodemographic characteristics while the second assessed other risk factors. Anonymity was maintained by using serial numbers.

2.6Ethical Consideration

Ethical approval was obtained from Rivers State Health Research Ethical Committee with REC number RSUTH/REC/202319 before commencement of this study. Verbal and written (questionnaire) consent were also obtained from subjects before samples were collected.

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2.7 Specimen Collection and Processing

Two hundred and ten (210) stool samples were collected in sterile well-labelled universal bottles and placed in sterile ziploc bags and taken immediately to the microbiology laboratory for analysis. Subjects were given proper guidance for collecting the stool samples. Where delay was inevitable, samples were stored in the refrigerator at 4°C. Two hundred and 10 (210) food samples were collected in sterile containers to avoid contamination. Six different food categories (Sea food, n = 35; Chicken, n = 35; Beef and Pork, n = 35, Dairy products, n = 35, Vegetable and fruit, n = 35, Grains, n= 35) were sampled. The food samples were food that was commonly consumed in Port Harcourt metropolis.

2.7.1 Stool Culture

Stool samples were inoculated into freshly prepared Selenite F Broth and incubated at 37°C for 18 – 24 hours. The overnight culture was sub-cultured unto *Salmonella-Shigella* Agar (SSA) and Bismuth Sulfite Agar (BSA) and incubated for 18-24 hours at 35 – 37°C. Transparent colonies with black centers on SS agar and colonies with metallic sheen and black center on BSA were identified as presumptive *Salmonella.*

2.7.2Food Culture

Salmonella was isolated based on standard protocols [28]. A 1:10 dilution of each food sample was made by weighing 10g of the food sample and grinding it using a small pestle and mortar before homogenization with 90ml peptone water. The homogenate was then incubated at 37 ℃ for 24 hours. Following incubation, 1ml of the culture was transferred

aseptically into 10 ml of sterile Selenite F Broth, mixed and then incubated at 37 ℃ for 24 hours. Following incubation, a loop-full of each culture was streaked unto the surface of recently prepared *Salmonella-Shigella* Agar (SSA) and Bismuth Sulfite Agar (BSA) and incubated at 37℃ for 24 to 48 hours and examined for the presence of *Salmonella* colonies.

2.7.3 Biochemical Identification

The biochemical characterization performed was based on standard techniques [28]. Suspected *Salmonella* colonies were picked from the agar plates and inoculated into the following Biochemical test tubes for confirmation; Triple Sugar Iron (TSI) test (Presumptive *Salmonella* colonies gave reactions typical of *Salmonella* by showing Alkaline/Acid with or without gas and hydrogen sulfide on TSI), Urease test (Presumptive *Salmonella* colonies were Urease negative), and Indole test (Presumptive *Salmonella* colonies gave negative Indole reaction). Colonies which gave all the reactions typical of *Salmonella* were kept in Nutrient Agar slants until further characterized

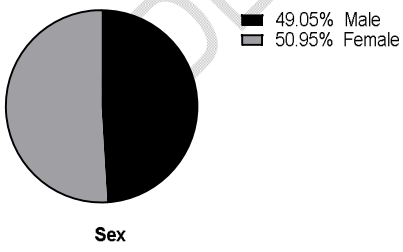
2.8 Data Analyses

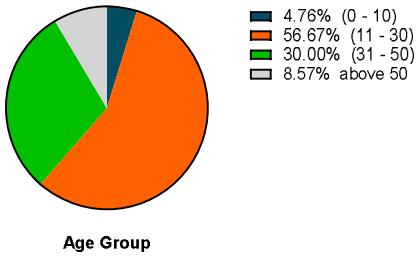
Raw data was collected using Microsoft excel and descriptive and inferential statistics were conducted using Graph pad prism version 8. Chi-square was utilized to identify statistically significant relationships between the prevalence of Salmonellosis and sociodemographic characteristics. The level of significance was defined as $P < .05$ at a 95% confidence interval.

3. RESULTS

3.1.1 Socio-demographics of the Study Participants

A total of 210 subjects aged between 4 to 69 (mean age = 29.8 years) were enrolled in this study. Most of the subjects were between the ages of 11 – 30 years (56.67 %), mostly females (50.95%), mostly students (48.10%), and had at least a tertiary school education (60%). Most of the subjects were residing in the Obio/Akpor local government area (57.14%) and were Christians (84.76%) (Figure 2).





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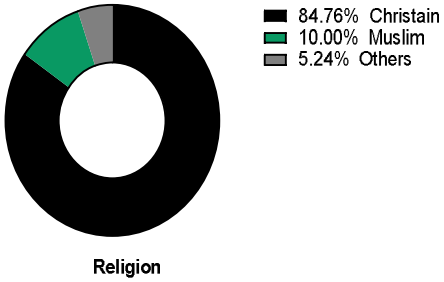

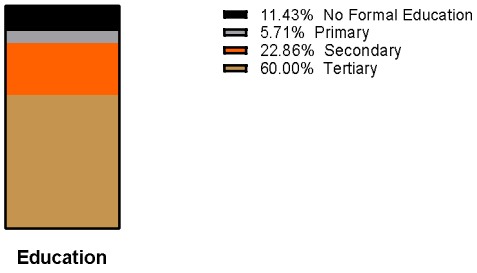

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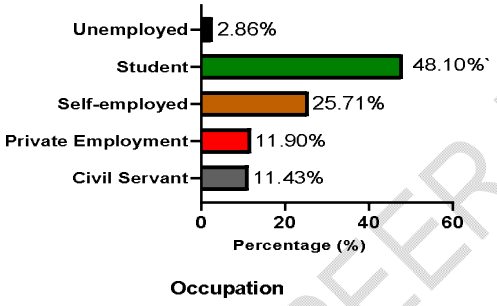

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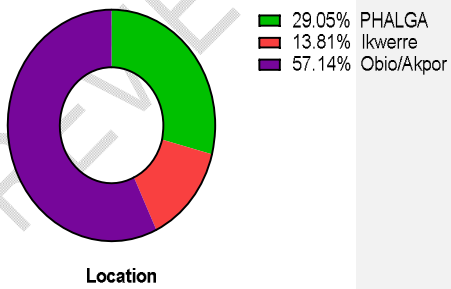

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Fig. 2. Sociodemographic characteristics of the stool-sampled population: A) Sex B) Age C) Religion D) Education E) Occupation F) Location

3.1.1 Characteristics of Food Samples

Two hundred and ten food samples were collected from different locations in the Port Harcourt metropolis. The food samples were grouped into five categories and were mostly chicken (21.9%), beef & pork (20%), fruits & vegetable (20%), dairy products (19.5%), and seafood (18.6%). The food samples were mostly gotten from the Obio/Akpor local government area (74.8%) (Figure 3).

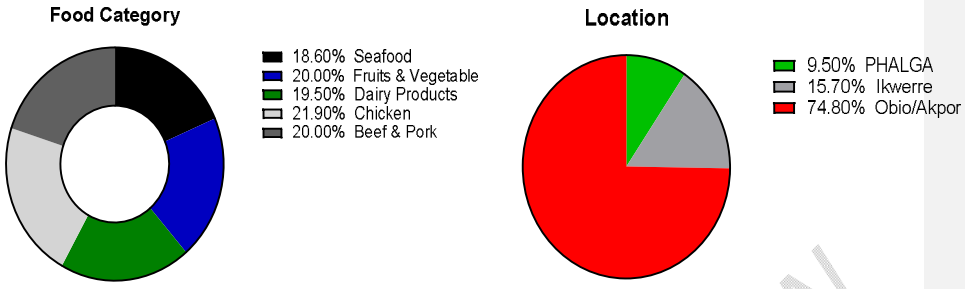

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Fig. 3. Food Sample Characteristics: A) Food Categories B) Location of Food Source

3.1.1 Prevalence of Non-Typhoidal *Salmonella* species

A total of 420 samples (210 stool and 210 food) were collected and tested for the presence or absence of *Salmonella* species. *Salmonella* was isolated from 12 (2.9%) of all samples tested. Of the 420 samples, 210 were stool samples from subjects in Port Harcourt metropolis of which five samples (2.4%) were confirmed to be *Salmonella* spp. and 210 were for food samples of which seven samples (3.3%) were confirmed to be *Salmonella* spp.positive by conventional microbiology method (Table 1).

Table 1.Prevalence of Non-Typhoidal *Salmonella* in Stool and Food Samples

Variable	Positive (%)	Negative (%)	Total (%)	X^2	Df	*P*-value
Food	7 (3.3)	203 (96.7)	210 (100)			
Stool	5 (2.4)	205 (97.6)	210 (100)			
Total	12 (2.9)	408 (97.1)	420 (100)	.3431	1	.56

3.1.1 Prevalence of Non-typhoidal *Salmonella* species in Stool Samples by Sociodemographic Characteristics

The Female subjects had a higher prevalence of 3 (3.7%) in comparison to their male counterpart, 1 (1.0%). Prevalence of *Salmonella* species for '0 – 10', '11 – 30', '31 – 50' and 'above 50' age groups were 1 (10%), 1 (0.8%), 1 (1.6%), and 2 (11.1%) respectively and was statistically significant (*P* = .02). The prevalence according to occupation were 0 (0%) for civil servants, 0 (0%) for the privately employed, 2 (3.7%) for self-employed, 1 (0.9%) for students and 2 (33%) for unemployed (*P*< .0001). Prevalence of *Salmonella* species for 'No formal education', Primary, Secondary, and Tertiary education were 2 (8.3%), 1 (8.3%), 2 (4.2%), and 0 (0%) respectively and was statistically significant (*P*< .03). For religion, Christian subjects had the highest prevalence 5 (2.8%) while Muslims and others were both 0 (0%). A similar case was observed in terms of location with Obio/Akpor having the highest prevalence with 5 (4.2%) while Ikwerre and PHALGA had 0 (0%) (Table 2).

Table 2. Prevalence of *Salmonella species* in Stool by Sociodemographic Characteristics

Variable	Positive (%)	Negative (%)	Total (%)	X^2	Df	p-*value*
Sex						
Male	1 (1.0)	102 (99.0)	103 (100)			
Female	4 (3.7)	103 (96.3)	33 (100)	0.729	1	.19
Total	5 (2.4)	205 (97.6)	210 (100)			
Age Group						
(0 – 10)	1 (10.0)	22 (91.7)	10 (100)			
(11 – 30)	1 (0.8)	118 (99.2)	119 (100)			
(31 – 50)	1 (1.6)	62 (98.4)	63 (100)	9.786	3	.02*
(above 50)	2 (11.1)	16 (88.9)	18 (100)			
Total	5 (2.4)	205 (97.6)	210 (100)			
Education						
NFE	2 (8.3)	22 (91.7)	24 (100)			
Primary	1 (8.3)	11 (91.7)	24 (100)			
Secondary	2 (4.2)	46 (95.8)	48 (100)	9.220	3	.03*
Tertiary	0 (0)	126 (100)	126 (100)			
Total	5 (2.4)	205 (97.6)	210 (100)			
Occupation						
Civil Servant	0 (0)	24 (100)	24 (100)			
Privately Employed	0 (0)	25 (100)	25 (100)			
Self-Employed	2 (3.7)	52 (96.3)	54 (100)	27.17	4	<.0001*
Student	1 (1.0)	100 (99.0)	101 (100)			
Unemployed	2 (33.0)	4 (67.0)	6 (100)			
Total	5 (2.4)	205 (97.6)	210 (100)			
Religion						
Christian	5 (2.8)	173 (97.2)	173 (100)			
Muslim	0 (0)	21 (100)	21 (100)	0.9208	3	.63
Others	0 (0)	11 (100)	11 (100)			
Total	5 (2.4)	205 (97.6)	210 (100)			
Location						
Obio/Akpor	5 (4.2)	115 (95.8)	120 (100)			
Ikwerre	0 (0)	29 (100)	29 (100)	3.841	2	.15
PHALGA	0 (0)	61 (100)	61 (100)			
Total	5 (2.4)	205 (97.6)	210 (100)			

3.1.1 Prevalence of Non-typhoidal *Salmonella* species in Food Samples

Two hundred and ten food samples (fruits & vegetables, Sea food, Dairy Products, Beef & Pork, and Chicken) were collected for culture with food in the chicken category having the highest prevalence with 4 (8.7%) followed by Seafood with 2 (5.1%), Dairy products with 1 (2.4%) while food in Beef and Pork category and Fruits and Vegetables both had 0 (0%). For the location, food collected from Obio/Akpor had a prevalence of 7 (4.5%) while Ikwerre and PHALGA had 0 (0%) each (Table 3).

Table 3. Prevalence of *Salmonella* in Food Samples and Sociodemographic Characteristics

Variable	Positive (%)	Negative (%)	Total (%)	X^2	Df	p-*value*
Location						
Obio/Akpor	7 (4.5)	150 (95.5)	157 (100)			
Ikwerre	0 (0)	33 (100)	33 (100)	2.536	2	.28
PHALGA	0 (0)	20 (100)	20 (100)			
Total	7 (3.3)	203 (96.7)	210 (100)			
Food Category						
Beef & Pork	0 (0)	42 (100)	42 (100)			
Chicken	4 (8.7)	42 (91.3)	46 (100)			
Dairy Products	1 (2.4)	40 (97.6)	41 (100)	7.493	4	.11
Fruits & Vegetable	0 (0)	42 (100)	42 (100)			
Seafood	2 (5.1)	37 (94.9)	39 (100)			
Total	7 (3.3)	203 (93.7)	210 (100)			

* Statistical significance $P < .05$; Values in parenthesis = percentages

3.1.1 Prevalence of *Salmonella* species by Month

The stool and food samples were collected during the same period (December 2022 to November 2023). There were more positive samples for the stool samples during the month of March (2) with February, April and June having 1 each. Fig. 3. and for Food samples, there were more positive samples in the months of January, March and April (two each) while February had one positive sample. Fig 3.

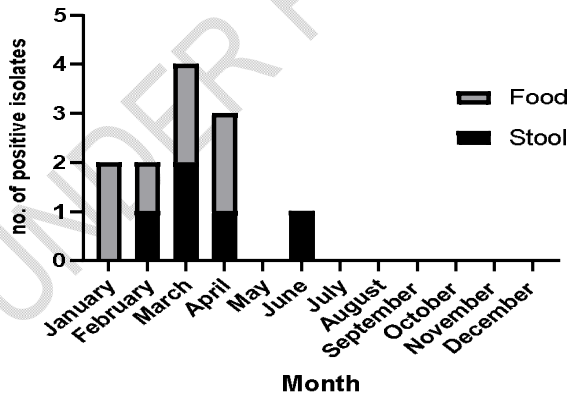

Fig. 4 Positive *Salmonella* samples by month.

3. DISCUSSION

Salmonella infection is very common in developing countries especially the non-typhoidal species. The common vehicle of transmission is via food. The present study investigated the prevalence of non-Typhoidal *Salmonella* in food sources and human stools using different parameters such as seasons, sociodemographic factors and type of sample (clinical and non-clinical). Four hundred and twenty (420) samples yielded 12 (2.9%) *Salmonella* isolates. *Salmonella* was positive in 5 (2.4%) of human samples (stool) and 7 (3.3%) of food samples. This higher prevalence in food samples in comparison to stool samples is in accordance with a study carried out by Ndu *et al*. [29] who also reported a higher prevalence in food samples. This may be due to the fact that animals from which food are gotten is the main reservoir for *Salmonella* and an immuno-competent human body can fight off *Salmonella* infections easily. The 3.3% prevalence rate from food sources reported in this study is lower than that reported by Ndu *et al*. [29] with 8.2% from ready-to-eat food samples. A similar study on isolation of *Salmonella* from raw beef and chicken used in Abuja fast-food restaurants by Bawa *et al.* [30] reported a prevalence of 1.5% which is lower than that reported in the present study. Konne *et al.* [31] showed a *Salmonella* species was the second most prevalent pathogen in roasted beef with a prevalence of 17% which is much higher than that obtained in this study. This difference in prevalence may be due to the location of sampling with restaurant's food likely to be more hygienic compared to road-side food.

The 2.4% prevalence in human (stool) samples in this study is higher than that reported by Akinyemi *et al*. [27] that demonstrated a 0.9% prevalence in humans living in Lagos. A similar study by Aworh *et al*. [32] on rare serovars of non-typhoidal *Salmonella* in abattoir workers reported a prevalence of 4.2% which is higher than the 2.4% prevalence rate in humans gotten in the present study. This variance in prevalence rates may be due to differences in sample sizes, the unsanitary nature of abattoirs and cattle in the abattoirs have been implicated as a likely source in the transmission of non-typhoidal *Salmonella* to humans.

Previous studies have suggested possibility of association between age and infection [33]. The age groups with the highest prevalence were the '0 – 10' age group with a 10% prevalence and 'above 50' age group with 11.1% prevalence and the difference was statistically significant compared to other age groups (*P* value = .02). These findings are consistent with a study by Zaidenstein *et al*. [34] who reported a significant difference in Israeli patients <10 and ≥60 years old. This may be due to the underdeveloped immune system in the '0 – 10' age group and the weakened immune system in the 'above 50' age group. The females had a higher prevalence (3.7%) compared to the male (1%). This is consistent with the findings of Kebede *et al*. [35] that reported a higher *Salmonella* prevalence among female diarrheic patients in Ethiopia. In terms of educational status, there was a higher prevalence in subjects with non-formal education (8.3%) and those with only primary education with a significant difference compared to those in other groups (*P* value < .03). This may be due lack of knowledge on proper food handling. Also, there was a significant difference in prevalence between the occupational classes with the unemployed (33%) having the highest prevalence while the civil servants and privately employed had a zero percent (0%) prevalence. This may be down to accessibility to properly cooked food and also due to the fact that those in the unemployed category were also children less than 5 years and adults older than 65 years both of which do not have a suitable immune system to fight off infections. In terms of religion, the Christians had the highest prevalence but there was no significant difference in comparison to other religions.

The months with the highest prevalence were between January and June with March (14.3%) having the highest prevalence for the stool isolates and April (25%) having the

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highest prevalence for food isolates. This may be due to the warm nature of these months in Nigeria and warmer weather create ideal conditions for *Salmonella* to grow [36]. In terms of location, the Obio/Akpor had a higher prevalence rate in both stool and food samples compared to the two other local governments, but the difference was not statistically significant. For the food samples, food in the chicken category had the highest prevalence (8.7%) compared to other food category which were beef & pork (0%), dairy products (2.4%), fruits & vegetables (0%) and seafood (5.1%). This was contrary to a study carried out by Zaidi *et al*. [37] which reported a higher prevalence in beef and pork samples.

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4. CONCLUSION

The present study shows that the prevalence of non-typhoidal *Salmonella* was 2.9%, with the age, educational background and occupation of the status being significantly associated ($P < .05$) with the prevalence of the infection. It also demonstrated that this pathogenic bacterium is found more commonly in food (3.3%) especially in chicken (8.7%) in comparison to stool (2.4%). The study also shows that the infection is more common during the months of January to June which are the warmest months in Nigeria. Health campaigns should be carried out to enlighten the populace about the risks involved in the prevalence of this infection so as to reduce its burden.

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CONSENT

All authors declare that 'written informed consent was obtained from the patient. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (Rivers State Health Research Ethical Committee with REC number RSUTH/REC/202319) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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