Prevalence of *Salmonella enterica* Serovar Typhi among Patients attending Selected Health Care Centres in Lafia, Nasarawa State, Nigeria

Terna, F.C[1]., Chuku, A[1]. and Obiekezie, S.O[2].
[1] Department of Microbiology, Federal University Lafia, PMB 146 Lafia Nasarawa State, Nigeria.
[2] Department of Microbiology, Nasarawa State University Keffi.
Corresponding author: paulternafaith@gmail.com; Tel: 07030350853

Abstract: A study was carried out to investigate the prevalence of *Salmonella enterica* serotype Typhi among 300 patients attending selected health care centres in Lafia, Nasarawa State Nigeria. Demographic data of patients were collected via semi-structured questionnaire. Bacterial isolates from stool samples collected from patients were identified using standard biochemical and serotyping methods. Stool samples of 17 (5.70%) patients tested positive for *Salmonella* Typhi, and strains having the somatic O antigen were more prevalent (88.24%) than those with the flagellar H antigen (11.76%). Higher prevalence of *Salmonella* Typhi was observed in females (7.05%) compared to males (4.17%), and in adults (5.74%) compared to children (5.50%). Prevalence was also higher in singles (7.39%) compared to married (4.00%) and in non-literate patients (11.91%) compared to literates (4.65%). Higher prevalence values were observed among retirees (50%) compared to civil servants (5.81%), private sector workers (5.17%) and unemployed (5.20%). Higher prevalence values were observed among semi-urban dwellers (8.80%) compared to urban dwellers (3.19%) and rural dwellers (5.56%). The major source of drinking water of patients was bore hole (85.67%). There was no significant association between all demographic categories of patients under investigation and infection by *Salmonella* Typhi (P>0.05). Improved personal hygiene, supply of potable drinking water, targeted vaccination, and regular screening and licensing of public food and water vendors, are recommended for the control of typhoid fever caused by *Salmonella enterica serovar* Typhi, in the studied area.

Key Words: Health care centres, Prevalence, Patients, Risk factors, *Salmonella enterica serovar* Typhi.

INTRODUCTION

Salmonellae that cause significant human diseases are classified as *Salmonella enterica,* which currently consists of approximately 2,587 serotypes (Grimont and Weill, 2007). They produce three main types of diseases in humans; enteric fevers (typhoid and paratyphoid fevers), bacteremia with focal lesions, and gastroenteritis, of which enteric fever is most prevalent worldwide, resulting in about 600,000 deaths annually (Gautam *et al.,* 2002).

Typhoid fever, a severe systemic illness caused by *Salmonella enterica* serotype Typhi, is an important public-health problem in many developing countries, where it is responsible for about 21.6 million illnesses, resulting in 216,500 deaths every year (Wang *et al.,* 2010), and contributing negatively to economic growth, due to the cost of surveillance investigation, treatment, and prevention of illness (Pui *et al.*, 2011). Enteric fevers caused by *S.* Typhi and *S.* Paratyphi are not only endemic in Nigeria, but constitute a great socio-medical problem (Zailani *et al.*, 2004; Abdullahi, 2010), being responsible for many cases of pyrexia of unknown origin (Akinyemi *et al.,* 2000), and high morbidity and mortality (Ekenze *et al.*, 2008; Nasir *et al.*, 2008; Effa and Bakirwa, 2008). Major symptoms of the infections include persistent high fever with low pulse rate, severe headache, nausea, mental confusion, abdominal tenderness and pain (Umeh and Agbulu, 2009).

The objective of this study is to evaluate the prevalence of *Salmonella* Typhi in patients attending selected heath care centres in Lafia, Nasarawa State, Nigeria. Findings on the prevalence pattern of *Salmonella* Typhi of *Salmonella* Typhi reported in this study shall be useful in the prevention and control of typhoid and enteric fevers in the studied area.

MATERIALS AND METHODS

a). Sample Collection

A total of 300 stool samples were randomly collected from patients undergoing medical examination in the Out-Patient Department (OPD) of three (3) health care centers in Lafia, Nasarawa State, using sterile plastic containers.

Data such as age, gender, marital status, occupation, educational qualification, and source of drinking water were collected from patients via semi-structured questionnaire. Determination of sample size was carried out using the minimum sample size calculation formula reported by Charan and Biswas (2013) as follows:

$n = Z^2IT/p^2$

Where, n = Sample Size

Z = 1.96 (Constant at 95% Confidence Interval)

I = Expected incidence (26.5%) (Enabulele and Awunor, 2016)

T = 1-I

p = Precision (Usually 0.05 at 95% Confidence Interval)

$$n = \frac{1.96^2 \times 0.265\ (1-0.265)}{0.05^2} = 299.17$$

Samples were cultured in selenite F broth (Difco) and incubated for 24 h at 37^0C and sub-cultured on *Salmonella-Shigella* agar (Oxoid) for 24 h at 37^0C. Presumptive *Salmonella* colonies from positive plates were transferred into Brain Heart Infusion agar (Oxoid) and identified using biochemical techniques such as TSI, oxidase test, catalase test and indole test (Aryal, 2015).

b). Serotyping of *Salmonella* isolates

The method of Grimont and Weill (2007) was adopted, where a loop full of bacterial cells was transferred from nutrient agar and emulsified in a drop of normal saline on a sterile ceramic tile, rocked gently and observed for the presence or absence of agglutination. In the absence of agglutination in the preceding step, a drop of either the somatic antigen (O) or the flagellar (H) (Oxoid) was separately added and rocked gently for 10 seconds. The occurrence of agglutination was indicative of a positive reaction.

c). Data Analysis

Data obtained from the study was subjected to Chi square analysis and Fisher's exact test to test for strength of association at 5% level of probability, using SPSS statistical software version 22.

RESULTS

Differences in demographics and prevalence of *Salmonella* Typhi were observed among the 300 sampled patients (Table 1). 50.00% of the patients were married, female (52.00%), above 18 years of age (69.67%) and literate (86.00%). Patients were mostly unemployed (51.33%) and resident in urban areas (52.33%). The major source of drinking water of patients was bore hole (85.67%), and 66.67% had a history of previous treatment for typhoid fever. Higher prevalence of infection by *Salmonella* Typhi was observed in females (7.05%) compared to males (4.17%), and in adults (5.74%) compared to children (5.50%). Prevalence was higher in singles (7.39%) compared to married (4.00%) and in non-literate patients (11.91%) compared to literates (4.65%). Higher prevalence values were observed among retirees (50%), semi-urban dwellers (8.80%), persons with no previous typhoid treatment history (6.60%) and persons who had well water as their primary source of drinking water (13.33%). There was no significant association between all demographic categories of patients under investigation and infection by *S.* Typhi ($P>0.05$). Out of 56 suspected *Salmonella* isolates obtained from 300 patients (Table 2), 25 isolates representing 8.33% of total sample population tested positive for *Salmonella* by reacting negatively to Gram's stain, oxidase, and indole. *Salmonella*-positive isolates also reacted positively to catalase and Triple Sugar Iron (TSI) tests.

Seventeen (17) out of the 25 *Salmonella*-positive isolates, tested positive for *Salmonella* Typhi by reacting positively to either O or H antigens (Table 3). *Salmonella* Typhi strains with the O antigen were most prevalent (88.24%) compared to those with the H antigen (11.76%).

Table 1. Prevalence of *Salmonella* Typhi among Different Demographic Categories of Patients

Factor	No. Observed	Frequency (%)	No. Infected	Prevalence (%)	Chi-square (χ^2) value	P value
Gender						
Male	144	48.00	6	4.17	1.17	0.28
Female	156	52.00	11	7.05		
Age (years)						.
Children (Below 18)	91	30.33	5	5.50	0.01	0.93
Adults (Above 18)	209	69.67	12	5.74		
Marital Status						
Single	149	49.67	11	7.39	1.60	0.21
Married	150	50.00	6	4.00		
Divorced	00	0.00	00	0		
Widowed	01	0.33	00	0		
Educational Status						
Non-Literate	42	14.00	5	11.91	NA	0.72[F]
Literate	258	86.00	12	4.65		
Occupation						
Civil Service	86	28.67	5	5.81	7.45	0.06
Private Service	58	19.33	3	5.17		
Retiree	02	0.67	1	50.00		
Unemployed	154	51.33	8	5.20		

[F] = P value using Fisher's Exact Test
NA = Not Applicable

Table 1. (Continued)

Factor	No. Observed	Frequency (%)	No. Infected	Prevalence (%)	Chi-square (χ^2) value	P value
Residence						
Urban	157	52.33	5	3.19	4.12	0.13
Semi-urban	125	41.67	11	8.80		
Rural	18	6.00	1	5.56		
Typhoid Treatment History						
Treated Before	200	66.67	11	5.50	0.03	0.86
Never Treated Before	100	33.33	6	6.00		
Sources of Drinking Water						
Bore hole	257	85.67	15	5.45	NA	0.24[F]
Well	15	5.00	2	13.33		
Bottled water	3	1.00	0	0.00		
Satchet water	23	7.67	0	4.35		
River	1	0.33	0	0.00		
Breast milk	1	0.33	0	0.00		

[F] = P value using Fisher's Exact Test
NA = Not Applicable

Table 2. Biochemical Characteristics of Suspected *Salmonella* Isolates From Patient's Stool

S/No.	Isolate No.	Biochemical Characteristics					
		Gram Stain	Oxidase	Catalase	Indole	Triple Sugar Iron Test (TSI)	*Salmonella* sp.
1	007	-	-	+	-	+	+
2	016	-	-	+	+	+	-
3	018	-	-	+	-	+	+
4	019	-	-	+	-	+	+
5	021	-	-	+	-	-	-
6	022	+	+	-	-	+	-
7	024	-	-	+	+	-	-
8	026	-	-	+	-	+	+
9	028	-	-	+	-	+	+
10	030	+	+	-	-	+	-
11	032	+	-	-	-	+	-
12	041	-	-	+	-	+	+
13	047	-	-	+	-	+	+
14	048	-	-	+	-	-	-
15	051	-	-	+	+	-	-
16	053	-	-	+	-	-	-
17	054	-	-	+	+	+	-
18	058	-	-	+	-	+	+
19	090	-	-	+	-	+	+
20	093	-	-	+	-	+	+
21	095	+	+	+	+	-	-
22	101	-	-	+	-	+	+
23	105	-	-	+	-	+	+
24	111	+	+	-	+	-	-
25	112	-	-	+	-	+	+
26	116	-	-	+	-	+	+
27	119	-	-	+	-	+	+
28	132	+	+	-	+	+	-
29	138	-	-	+	-	+	+
30	142	-	-	+	-	+	+
31	152	+	-	+	+	-	-
32	153	-	-	+	-	+	+
33	199	-	-	+	-	-	-
34	200	-	-	+	-	+	-
35	204	-	-	+	-	-	-
36	216	-	-	+	-	+	+
37	217	-	-	+	-	-	-
38	220	-	-	+	-	-	-
39	221	-	-	+	-	+	+
40	222	-	-	+	+	-	-
41	223	-	-	+	+	-	-
42	237	-	-	+	-	-	-
43	243	-	-	+	-	-	-
44	247	-	-	+	-	-	-
45	265	-	-	+	-	+	+
46	267	-	-	+	-	+	+
47	272	-	-	+	+	-	-
48	273	-	-	+	+	+	-
49	274	-	-	+	-	+	+
50	292	-	-	+	-	+	+
51	293	-	-	+	-	+	+
52	294	-	-	+	+	-	-
53	295	-	-	+	+	-	-
54	296	-	-	+	+	-	-
55	297	-	-	+	-	-	-
56	298	-	-	+	+	-	-

Table 3. Serotype Characteristics of *Salmonella* Isolates Collected From Patients' Stool

		Antigens	
S/No.	Isolate No.	H	O
1	007	-	+
2	018	-	+
3	019	-	+
4	026	-	+
5	028	-	+
6	041	-	+
7	047	-	+
8	058	-	+
9	090	-	+
10	093	-	-
11	101	-	+
12	105	-	-
13	112	-	+
14	116	-	-
15	119	-	+
16	138	-	+
17	142	-	+
18	153	-	-
19	216	-	-
20	221	-	-
21	265	+	-
22	267	+	-
23	274	-	-
24	292	-	+
25	293	-	-

DISCUSSION

Salmonella Typhi was observed in stool samples of patients in the present study. Prevalence of suspected *Salmonella serovars* in this study were however less than previous reports of Abioye *et al.* (2017) in Karu, and Gbodo and Anumudu (2019) in Owerri, Nigeria. Variations in prevalence of typhoidal and non-typhoidal *Salmonellae* are a function of the epidemiological dynamics operational in a given area at a given time. Differences in such conditions as the availability of basic infrastructure, literacy levels, level of community hygiene and other economic determinants could have accounted for the observed disparity in prevalence values.

Salmonella enterica serovar Typhi strains with the O antigen were most prevalent compared to those with the H antigen. This is at variance with the reports of Feasey and Gordon (2014) who mentioned that in endemic countries, the population has higher in H antibody titres. The increase in prevalence of *S.* Typhi carrying the O antigen as opposed to H antigen could be an indication of a possible phenotypic transition currently obtainable among *S.* Typhi populations in the study area. This is however subject to further investigation.

Higher prevalence of *S.* Typhi infection was observed in females compared to males. Similarly, Ogah *et al.* (2015) in a study on prevalence of salmonellosis among food handlers, and the health implications on the food consumers in Lagos State, Nigeria, reported that more females than males were infected with *Salmonella enteria serovar* Typhi, while Ajayi *et al.* (2015) and Ezeigbo *et al.* (2015), reported higher prevalence in males compared to females.

The factors that predispose persons to infection by *Salmonella* Typhi vary from one geographical location to another. The higher prevalence of *S.* Typhi in female patients could be a result of increased levels of background exposure to infection probably through consumption of infected water and food. This is however subject to further investigation.

In the present study, higher prevalence of *S.* Typhi infection was observed in adults, compared to children. A similar observation was made by Ezeigbo *et al.* (2015) in Aba, Southeastern Nigeria. On the contrary, Ochiai *et al.* (2008) reported an inverse correlation between typhoid incidence and the mean age of typhoid cases in five Asian countries. Adults compared to children are likely to be more predisposed to infection by *S.* Typhi through frequent predisposing habits such as eating hawked foods and drinking water from random sources. This also explains the higher *S.* Typhi prevalence observed in singles who are mostly fond of eating foods in restaurants and eateries, compared to married responds who in most cases prefer to eat home-made foods prepared under better hygienic conditions. In same vein, Agbakwuru *et al.* (2003) mentioned that eateries and public eating places are the commonest means of typhoid fever spread.

Non-literate patients and retirees had higher incidence of *S. enterica serovar* Typhi infection compared to literates. The low educational background of the non-literate patients could mean that they may have little or no understanding of food safety and personal hygiene as well as the risk of microbial or chemical contamination of food and how to avoid them. Similarly, Gasem *et al.* (2001) stated that being unemployed or having a part-time job was associated with an increased risk for typhoid fever. Retirees in the study area represent persons mostly aged between 60 years and above, and signifies the age group with reduced health immunity and high economic dependence ratio. They are thus more likely to eat foods prepared and served to them by others rather than foods cooked by themselves, and so are more at risk of infection by *S.* Typhi.

Semi-urban dwellers and persons who reported well water as their primary source of drinking water had the highest comparative prevalence of *S.* Typhi. Higher incidence of salmonellosis in semi-urban and rural areas has been linked to lack of portable water, inadequate sewage disposal, flooding, and lack of personal hygiene (WHO, 1998). Although, a direct link between well water and typhoid fever infection may be difficult to establish, possible feacal contamination of most well waters could be a major contributory factor to the high infection frequency.

CONCLUSION

The findings of this study showed the relationship between demography and prevalence of *Salmonella* Typhi among patients attending different health care centres in Lafia. Prevalence of *S.* Typhi was higher in adult females, retirees, non-literate, and semi-urban dwellers, who used well water as primary source of drinking water. Improved personal hygiene, supply of potable drinking water, targeted vaccination, and regular screening and licensing of public food and water vendors, are recommended for the control of typhoid fever caused by *Salmonella enterica serovar* Typhi, in the studied area.

REFERENCES

Abdullahi, M. (2010). Incidence and antimicrobial susceptibility pattern of *Salmonella* species in children attending some hospitals in Kano Metropolis Kano State – Nigeria. *Bayero Journal of Pure and Applied Sciences*, 3(1): 202-206.

Abioye, J., Adiuku, B., and Adogo, L. (2017). The prevalence of typhoid fever in Bingham University. *GSC Biological and Pharmaceutical Sciences,* 1(3): 37-43.

Agbakwuru, E.A., Adesunkanmi, A.R., Fadiora, S.O., Olayinka, O.S., Aderonmu, A.O., and Ogundoyin,

O.O.A. (2003). Review of typhoid perforation in rural African Hospital. *West African Medicine,* 22(1):22-25.

Ajayi, O.E. Olukunle, O.F., and Boboye, B.E. (2015). Prevalence of Typhoid Fever among Different Socio-demographic Groups in Ondo State, Nigeria. *Journal of Applied Life Sciences International,* 3(2): 89-95.

Akinyemi, K.O., Coker, A.O., Olukoya, D.K., Oyefolu, E.O., and Amorighoye, E.P. (2000). Prevalence of multidrug resistant *Salmonella typhi* among clinically diagnosed typhoid fever patients in Lagos, Nigeria. *Zeitschrift für Naturforschung,* 55:489-493.

Aryal, S. (2015). Biochemical test and identification of *Salmonella Typhi. A*vailable online at: https://microbiologyinfo.com/biochemical-test-and-identification-of-salmonella-typhi/. Accessed: 31/12/2017.

Charan, J., and Biswas, T. (2013). How to calculate sample size for different study designs in medical research. *Indian J. Psychol. Med*., 35:121-6.

Effa, E.E., and Bukirwa, H. (2008). Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cochrane Database of Systematic Reviews*, (4):1-2.

Enabulele, O., and Awunor, S. N. (2016). Typhoid fever in a tertiary hospital in Nigeria: Another look at the Widal Agglutination Test as a preferred option for diagnosis. *Nigerian Medical Journal*, 57(3), 145.

Ezeigbo, O.R., Agomoh, N.G., and Asuoha-Chuks N. (2015). Laboratory Diagnosis of Typhoid Fever using Widal and Blood culture Methods in Aba, Southeastern Nigeria. *American Journal of Microbiological Research,* 3(6): 181-183.

Ekenze, S.O., Okoro, P.E., Amah, C.C., Ezike, H., and Ikefuna, A.N. (2008). Typhoid ileal perforation: analysis of morbidity and mortality in 89 children. *Nigerian Journal of Clinical Practice*, 11 (1):58-62.

Feasey, N.A. and Gordon, M.A. (2014). *Manson's Tropical Infectious Diseases*, Twenty-Third Edition. Elsevier LTD. Pp. 37–348.

Gasem, M.H., Dolmans, W.M.V., Keuter, M., and Djokomoeljanto, R. (2001). Poor food hygiene and housing as risk factors for typhoid fever in Semarang, Indonesia. *Tropical Medicine and International Health,* 6(6): 484-490.

Gautam, V., Gupta, N.K., Chaudhary, U., and Arora. D.R. (2002). Sensitivity Pattern of *Salmonella* serotypes in Northern India. *The Brazilian Journal of Infectious Diseases*, 6(6): 281-287.

Gbodo, E.E., and Anumudu, C.K. (2019). Prevalence of *Salmonella typhi* infection among food handlers in Imor State University Owerri Nigeria and its environs. *Journal of Tropical Diseases,* 7:4.

Grimont, P.A.D., and Weill, F.X. (2007). Antigenic formulae of the *Salmonella* serovars, *WHO Collaborating Center for Reference and Research on Salmonella,* 9(150): 1-166.

Nasir, A.A., Adeniran, J.O., Abdur-Rahman, L.O., Odi, T.O., and Omotayo, J.A. (2008). Typhoid intestinal disease: 32 perforations in 1 patient. *Journal of Nigeria Postgraduate Medicine,* 15:55–57.

Ochiai, R.L., Acosta, C.J., Danovaro-Holliday, M.C., Baiqing, D., Bhattacharya, S.K., Agtini, M.D., and Clemens, J.D. (2008). A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bulletin of the World Health Organization*, 86(4): 241-320.

Ogah, J.O., Adekunle, O.C., and Adegoke, A.A. (2015). Prevalence of Salmonellosis among Food Handlers and the Health Implications on the Food Consumers in Lagos State,

Nigeria. J. Med. Microb. Diagn., 4:187.

Pui, C.F., Wong, W.C., Chai, L.C., Tunung, R., and Jeyaletchumi, P. (2011). *Salmonella*: A food borne pathogen. *International Food Research Journal,* 18: 465-473.

Umeh, E., and Agbulu, C. (2009). Distribution Pattern of *Salmonella* Typhoidal Serotypes in Benue State Central, Nigeria. *The Internet Journal of Epidemiology,* 8(1):1-7.

Wang, S., Singh, A.K., Senapati, D., Neely, A., and Yu, H. (2010). Rapid colorimetric identification and targeted photothermal lysis of *Salmonella* bacteria by using bioconjugated oval-shaped gold nanoparticles. *Journal of Chemistry,* 16(19): 5600-5606.

WHO (1998). Vaccination against Typhoid Fever, Present Status. World Health Organization, Geneva Switzerland.

Zailani, S.B., Aboderin, A.O., and Onipede, A.O. (2004). Effect of socio-economic status, age and sex on antibody titre profile to Salmonella typhi/paratyphi in Ile-Ife, Nigeria *Nigerian. Journal of Medicine,* 13 (4):383-7.