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Prevalence of salmonella agglutinins among patients attending hospitals in biu, Borno State, Nigeria

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ABSTRACT

The prevalence of Salmonella typhi and paratyphi A, B and C were investigated in blood samples of patients attending General Hospital and two other clinics in Biu Local Government area of Borno State, Northeastern Nigeria. A total of 204 blood samples, from 90 (44.1%) males and 114 (55.9%) females were collected from patients, within a two month period. Widal test was used to detect the antibody titers in sera, obtained by centrifugation at 3000rpm for 5 minutes. About (39) 43.3% males and (45) 39.5% females were positive. Higher numbers of positive cases were recorded for patients within 13-24 (45.9%) and 25-46 (42.9%) age ranges, but no case was reported for patients ≥ 68 years of age (n=2). Based on occupation, students/pupils have the highest (68.2%) antibody titers among different groups. More positive samples were detected in unmarried patients (47.5%), compared to married ones (36.6%), even though, there is no statistical significance (P > 0.05). About 27% of the samples showed the presence of group O antigens. This was followed by Salmonella paratyphi B (25%), Salmonella typhi (20.6%) and Salmonella paratyphi C (13.7%). For the H antigens however, they were in the order: Salmonella paratyphi B (25%) > Salmonella paratyphi C (19.6%) > Salmonella paratyphi A (18.6%) ≥ Salmonella typhi (18.6%).

Key words: *Salmonella*, Prevalence, Antibody titer, Enteric fever

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INTRODUCTION

Salmonella species are the main causes of enteric or typhoid fever, that poses health threat in developing countries. The disease has been described as endemic in tropical and sub tropical countries, with estimated annual incidences of 540 per 100,000 [1]. The world wide incidence was estimated to reach up to 17 million cases [2] and about 600,000 deaths per annum [3]. Areas with high disease burdens include South and East Asia, Africa south of the Sahara, and Latin America. This situation is attributed to rapid population growth, increased urbanization, inadequate sanitation, and insufficient water supply and crowding in homes and settlements. The situation is worsened by an overburdened health care system and an increasing number of people with HIV/AIDS [4].

Transmission of the infection is by faecal-oral route, when contaminated food or water is consumed or from contaminated hands [5]. The incubation period may be difficult to determine due to insidious onset, and mild or atypical clinical picture. However it ranges usually from 8–14 days [4]. *S. paratyphi* may cause *Salmonella* gastroenteritis resembling non-typhoid *Salmonella*, and in this case the incubation period is 1–10 days. Clinical

symptoms are non specific, but may range from headache to severe complications like haemorrhage and perforation of the intestine, the recognition of which may be difficult [4]. Death may result due to destruction of the intestine, bone marrow or other organs [6]. Antibiotic treatments have reduced the infection burden over the years especially in developed countries [7], despite the fact that resistance is very common [8].

Detection of typhoid bacteria depends on the collection of appropriate samples from patients in the laboratory. Viable organisms can be detected from blood, stool and urine cultures, depending on the period of exposure to the pathogens [5]. Serological (widal) test to determine typhoid agglutinins is the commonly used diagnostic method due to its rapidness, affordability and easy to perform [9]. Widal test has been the only rapid diagnostic assay used in developing countries like Nigeria, despite the fact that its reliability is controversial [5]. The test is based on macroscopically visible agglutination reaction between antibodies present in the serum and somatic (O) and flagella (H) antigens of the salmonellae, available as coloured preparations [9].

Cases of enteric fever are been reported to hospitals on daily basis in Nigeria. The situation is very high in most communities in the rural areas, where sanitation and potable water are unavailable. The harsh economic climate has encouraged a cancerous rate of household production of various food products with the attendants being risk to public health. The present study reports the prevalence of the disease in Biu Local Government communities. It highlighted the extent to which individuals of different age, gender and occupational status among the studied population were infected. This is with a view to creating awareness among public, local and regional authorities to enable them take effective measures for prevention and control of the infection.

MATERIALS AND METHODS

Study area

The study was carried out among patients attending General Hospital Biu and two other clinics in Biu, the capital of Biu Local Government, Borno State. The Local Government has a population of 176072 [10]. The major occupation of the people is farming. Local business and cattle rearing are also practiced to lesser extent. The whole of the Local Government headquarters and its environs lacks adequate pipe borne water. Most people use well and creek water for drinking and domestic purposes. Two hundred and four people were involved in the study, comprising of 90 males and 114 females.

Sample collection

About 3ml of blood samples were collected from each of the subject by venipuncture and transferred into sterile test tubes. They were centrifuged for 5 minutes at 3000rpm. The serum formed was separated from the packed cells into clean container in each case using pasture pipette. The samples were processed immediately, otherwise stored at 2 to 8^{o}C for later use.

Widal agglutination test

The sera obtained were diluted with normal saline to make 1:20 dilution. The antigen preparations (eight in number), were treated the same way. 20µl of the diluted sample was dropped on a test tile using micropipette and repeated eight times to correspond with the number of somatic (OA, OB, OC, and OD) and flagella (AH, BH, CH and DH) antigens. About 20µl of the antigens were added to respective serum samples on the test tile. The reactants were thoroughly mixed using separate applicator sticks in each case. The tile was rocked for 2 minutes, after which the result was read by observation of presence or absence of agglutination. The samples with positive result were selected for further analysis.

For confirmatory test, a method involving serial dilution in microtitre plate was employed for all positive samples. About 90µl and 50µl of normal saline were dispensed in to first and subsequent (2nd to 12th) wells of the microtittre plate respectively. To the 90µl normal saline, 10µl of the serum was added and mixed thoroughly. Serial dilution was carried out by taking 50µl from the 2nd up to the 12th well. 50µl of 1:20 dilution of antigens were added to microtiter wells containing the serially diluted sera accordingly. The preparations were mixed properly and incubated overnight. Antibody titers were read at the last well that shows agglutination after the period of incubation.

The result obtained was analyzed using statistical package (SPSS, version 12.0)

RESULTS

Out of the 204 samples processed, comprising of 90 and 114 male and female with different age and occupational status, 84 (41.2%) were positive with ≥1/160 antibody titer. The 43.3% (n=90) and 39.5% (n=114) of the male and female populations were positive for the salmonellae antibodies respectively. The tables below represent the data obtained from the experiment.

Table 1: Distribution of antibody titre against salmonella agglutinins based on sex

Species	Salmonella typhi				S. paratyphi A				S. paratyphi B				S. paratyphi C			
Antigen	O		H		O		H		O		H		O		H	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Male	22	24.2	20	22.2	28	31.1	18	20.0	24	26.7	19	21.1	1	12.2	18	20.6
Female	20	17.5	18	15.8	27	23.7	20	24.0	27	23.7	32	28.1	17	14,9	22	19.3
%	20.6		18.6		27.0		18.6		25.0		25.0		13.7		19.6	

Salmonella O and H agglutinins have been detected form the sampled population. Percentages of group O antigens were generally higher than those of H antigens in *Salmonella typhi* and *Salmonella paratyphi* A. For *Salmonella paratyphi* B, the percentages were the same (25%). In the case of *Salmonella paratyphi* C however, the percentage of group H antigens are higher than that of the O antigens.

Table 2: Distribution of salmonella infection base on sex

Sex	Number of sample tested	Number of positive	Percentage of positive
Male	90	39	43.3
Female	114	45	39.5
Total	204	84	41.2

(X-squared = 4e-04, df = 1, p-value = 0.9839)

The overall positive samples are 84 (41.2%), with males being predominant. Out of 114 females, 45 (39.5%) were positive for titers of 1/160 and above. The male were 39, making 43.3% of male's samples, even though, there is no statistical significance (P > 0.05).

Table 3: Distribution of salmonella infection based on age

Age group (Years)	Number of sample tested	Number of positive	Percentage of positive
1 – 12	43	17	39.5
13 – 24	61	28	45.9
25 - 46	63	27	42.9
47 – 68	23	7	30.4
>68	2	0	0.0
Unspecified	12	5	41.7
Total	204	84	41.2

(X-squared = 3.1804, df = 5, p-value = 0.6722)

The table shows the prevalence of serological reactivity to Salmonella antigen. Different age groups showed varying number of positive samples. Higher numbers were recorded in samples between 13 – 24 and 25 -46 years age range. Unspecified group showed less prevalence and none was recorded for elderly people above the age of 68, even though; there is no statistical significance (P > 0.05)

Table 4: Distribution of Salmonella infection based on marital

Marital status	Number of sample tested	Number of positive	Percentage of positive
Married	112	41	36.6
Single	80	38	47.5
Unspecified	12	5	41.7
Total	204	84	41.2

(X-squared = 1.8589, df = 1, p-value = 0.1728)

Unmarried people have higher prevalence of *Salmonella.* Out of the 80 samples tested, 47.5% were positive. This is followed by married people with 36.6% of 112 collected samples even though; there is no statistical significance (P > 0.05). Twelve peoples were unspecified for marital status and make up 41.2% out of 12 samples.

DISCUSSION

In this study, 204 blood samples were collected from patients attending hospital and two clinics in Biu, during a period of two months (August and September). Widal test was used to evaluate the presence of antibodies in the sera. A titer of 1/160 was considered positive for all the samples tested. The highest antibody titer recorded was that of *S. paratyphi* A and *S. paratyphi* B group O antigens, with 27% and 25% respectively. This corresponds with the work of Leon *et al*., [11], who reported higher rates of *S. paratyphi* A, compared to *S. typhi* in some Asian countries, but in contrast ton work of Shyamala *et al.* [12] who reported low prevalence of 8.57% in a tertiary care hospital in South India. The group H antibody titers of *S. paratyphi* B were relatively higher than the corresponding antibody titers of the other species or serotypes. 25% occurrence was also observed in this case. Other titers for the group O antigens were in the order: *S typhi* (20.6%) > *S. paratyphi* C (13.7%). The later is however infrequently reported in most cases of enteric fever. As for the remaining group H antigens, there were no much differences, as shown in table 1.

From the result outlined above, it is clear that incidences of typhoid fever are relatively high, although less; when compared to some instances reported in Nigeria. Studies on the prevalence of *Salmonella typhi* among patients in Abeokuta (south-western Nigeria) for example, reported 80.1% of 840 sampled population to be positive [1]. Out of the 90 males' samples used in this study, 39 (43.3%) were found positive for *Salmonella* agglutinins. The number of positive females samples however were 45 (39.5%) out of 114 as shown in Table 2, even though, there is no statistical significance (P > 0.05). This however indicated lower incidences compared to that of the male population. This finding agreed with that of Prajapati *et al*., [13], where incidences of the infection in Nepal were reported. The difference could be attributed to the fact that, outdoor activities are more pronounced in males than the females in most of rural communities like Biu, exposing them to high risk of infection.

In this study also, higher cases were recorded for 13 -24 years and adults of 25 – 26 years, where 45.9% (n=61) and 42.9% (n=63) samples were positive respectively. There exists no significant difference between the two age groups. However, a significant variation was noticed between the aforementioned age groups and people with ≥68 years of age; where 0.0% (n=2) was recorded. The number of children (1-3 years) with significant antibody titre was considerable as shown in table 3. This however, was precedented by the work of Prajapati *et al*., [13] in Nepal. This may be due to their immune status, unhygienic practices and high rate of exposure to contaminants.

Based on marital status, the prevalence is about 10% more singles (47.5%), than married (36.6%) individuals. The reason for this difference may be due to consumption of contaminated food or water by the unmarried people that often obtain their daily meals from street vendors. This is the common practice of bachelors in rural communities, especially when running their local businesses. It also buttressed the higher prevalence in males and adults that was shortly described above, due to apparent correlation. From table 4, it could be understood that no significant difference (P≥ 0.251) exists between most occupational statuses in relation to *salmonella* infection. However significant difference could be noticed when driving and schooling is considered, where 68.2% (n=22) and 0.0% (n=1) of incidences were recorded. The variation could however be affected by sample size of the later.

Although the study does not include source of drinking water as reference point, the prevalence is without doubt more or less related to consumption of contaminated water and food, owing to the fact that sources of potable water (e.g. tap water) are limited in the area. The two major sources of water used by the community are in no way free from contamination, making it unsafe for drinking, unless otherwise treated. The treatment may likely not be possible due, partly to ignorance and economic stress. Unsanitary practices by most food sellers are another contributing factor, as there are no health regulations governing their business. Inadequate treatment of acute illness is contributing significantly to the spread of the infection, as patients will continue to harbor and shade the organism for quite some times.

CONCLUSION

From our study presented here, it is clear that *Salmonella* agglutinins are traceable in patient at clinical presentation. The prevalence is of great public health concern, especially when such a disease is considered in 21st century, where emerging infections are becoming worrisome. Good sanitary practices are pertinent in limiting the spread of the infection. Provision of potable water and healthcare facilities by authorities at various levels is needed, in order to put a break to the endemic nature of the infection. Improvisation of safe drinking water by boiling is a duty of individuals, especially where proper means are impossible.

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