

**SEROPREVALENCE OF *SALMONELLA* AGGLUTININS AMONG
APPARENTLY HEALTHY STUDENTS OF A TERTIARY INSTITUTION IN
NORTH-EASTERN NIGERIA.**

ABSTRACT

Aim: To determine the level of *Salmonella* agglutinin and its titre among apparently healthy students of Tertiary Institution in North-eastern Nigeria.

Study Design: A cross-sectional study on Seroprevalence of *Salmonella* agglutinin;

Place and Duration of the Study: Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State, between September to December, 2016.

Methodology: This study is a cross sectional study in which 200 apparently healthy students of Federal Polytechnic Mubi were tested for *Salmonella* agglutinin using both slide and tube agglutination methods.

Statistical Analysis: Non parametric Mann-Whitney statistics and least significance difference (LSD) were used to test for significance difference in all the data obtained. Significance difference was taken when $P < 0.05$.

Results: The result showed that the number of males with positive Widal agglutinin was significantly higher than that of females ($P = 0.028$). The number of positive titre is significantly higher in age group 21-25 years ($P = 0.002$) when compared to other age brackets. However, there was no statistical difference between the age bracket 16-20 years and 31-35 years ($P = 0.747$). The result of the agglutination titre shows that more male had *Salmonella* titre for *Salmonella* Typhi O (81.8%) and *Salmonella* Typhi H (72.7%) while more female had *Salmonella* agglutinin titre for *Salmonella* Typhi H (44.4%) and *Salmonella* Paratyphi B-H (33.3%). It was also observed that only agglutinin for *Salmonella* Paratyphi A-O, B-O, C-O, B-H and C-H were present in the sera of male subjects up to the titre of 160 and at frequencies ranging from 1.6-58.8%. Furthermore, among females, it was observed that only agglutinins for *Salmonella* Paratyphi A-O, *Salmonella* Typhi C-H and *Salmonella* Typhi O and H were present in the sera of the subjects up to the titre of 160 and at frequencies ranging from 2.5-25.0%.

Conclusion: Detecting *Salmonella* agglutinin titre up to 160 among apparently healthy individuals is of public health significance. Thus, there is need to monitor this trend and create awareness on environmental and behavioural risk factors for *Salmonella* infections.

Keywords: Seroprevalence, *Salmonella*, Agglutinins, Apparently healthy.

INTRODUCTION

Typhoid and paratyphoid fever commonly called 'enteric fever' is caused majorly by *Salmonella* of the serotype Typhi and less commonly the serotype Paratyphi A, B or C. *Salmonella* is a Gram negative facultatively rod shaped members of the Enterobacteriaceae

family. They are non-spore forming with dimension of 0.7 – 1.5µm in diameter and 2 - 5µm in length and are mostly motile by means of peritrichous flagella [1]. Their complex antigenic structures (such as somatic O antigens, Capsular antigen and Flagella H Antigens) are often used for their classification.

In most developing countries especially in the tropics, typhoid fever is responsible for high rate of morbidity and mortality with an estimated annual global incidence of 21 million cases and more than 700, 000 deaths, while the less severe paratyphoid fever caused an estimated 5.4 million illness in 2000. The morbidity and mortality rate was however reported to be on the increase due to poor sanitation and hygiene, unavailability of vaccines and high cost of effective antimicrobials [2, 3].

Environmental and behavioural risk factors associated with typhoid fever includes eating unhygienic food, inadequate washing of hands before eating, drinking contaminated water that was not properly treated and handled, sharing and eating from the same utensils [4].

Typhoid and paratyphoid agents are transmitted majorly by faecal-oral route. The most important risk factors for the spread of this disease causing agents are apparently healthy carriers, convalescent and chronic carriers which usually shed the bacteria into the environment. Bacteria shed by these carriers contaminate food and water which further aid in the consistent spread of the organisms.

Most health facilities in developing countries like Nigeria relied on Widal test for diagnosing enteric fever [5]. This is because the test is simple, easy, readily available, inexpensive and relatively non-invasive; its diagnostic speed ensures prompt and adequate therapy for the disease [6]. The most prominent drawback of Widal agglutination test is cross-reactions. This occurs when antibody produced by non-typhoidal antigens reacts with typhoid specific – antigens [7]. Despite the drawback, if properly harnessed and interpreted accurately, Widal test still has significant diagnostic utility [8] in providing presumptive diagnosis for typhoid fever. Therefore, this study was carried out to determine *Salmonella* agglutinin and its titre among apparently healthy students of Federal Polytechnic Mubi.

MATERIALS AND METHODS

Study Area

The study area was Federal Polytechnic Mubi located in Mubi metropolis, Adamawa State, Nigeria. The institution was established by decree no. 33 of 1979 constitution. Mubi metropolis is a geo-political area comprising of two local government areas; Mubi North and Mubi South. The metropolis is located between latitudes 10° 05' and 10° 30'N of the equator

77 and between longitude 13° 12' and 13° 19'E of the Greenwich meridian. The two Local
78 government areas occupy a land area of 192,307 Km² and support a total population 260,009
79 people (National Population Census 2006). The area shares boundary with Maiha L.G.A in
80 the South, Hong L.G.A in the West, Michika L.G.A and Cameroon Republic in the East [9].

81 **Study Design**

82 This is a cross sectional study in which 200 apparently healthy students were randomly
83 recruited for the study.

84 **Sample Collection**

85 Blood samples from 200 apparently healthy students were collected randomly. The blood
86 samples were centrifuged in order to separate the serum from the plasma. The serums
87 collected were immediately used for detection of *Salmonella* agglutinin.

88 **Widal agglutination test**

89 SWE-CARE diagnostic febrile antigen kits (Spain) was used for the purpose of this test. The
90 rapid slide screening test was first carried out followed by the tube agglutination test
91 according to the manufacturer's specifications. The SWE-CARE diagnostic kit is suitable for
92 both rapid slide and tube agglutination test against human sera for the detection of *Salmonella*
93 agglutinins. The stained antigen suspensions are killed bacteria stained to enhance the reading
94 of agglutination test. The blue stained antigens are specific to the somatic 'O' antigens while
95 the red antigens are specific to the flagella 'H' antigens.

96 **Statistical Analyses**

97 Non parametric Mann-Whitney statistics and least significance difference (LSD) were used
98 to test for significance difference in all the data obtained. All statistical analyses were carried
99 out using SPSS version 17. Significance difference was taken when $P < 0.05$.

100 **Ethical Consideration**

101 Verbal informed consent of each participant was obtained prior to testing. Ethical approval
102 was also obtained from the institutions' Research and Seminar Committee Board. The
103 individual laboratory results were kept confidential and given to the participants at the
104 completion of the project.

105 **RESULTS**

106 Result from the tested 200 serum sampled, a significant number of 120 (60.0%) were found
107 to be Widal positive with at least one of the tested antigens (Table 1), although the results
108 showed that the percentage of Widal positive subjects was not significantly different from
109 Widal negative subject ($P = 0.221$). The result also showed that the number of males with

positive Widal agglutinin 81.8% (n=110) is significantly higher than that of females 33.3% (n=30) (P=0.028).

Table 2 showed the prevalence of Widal positive sera in relation to age group of the students. The age of the study population ranged from 16-40 years. The result showed that the age group 21-25 years had the highest (71.3%) Widal positive agglutinin titre, while the lowest agglutinin titre was recorded in the age group 36-40 years. Statistically, the number of positive titre is significantly higher in age group 21-25 years (P=0.002) when compared to other age brackets. However, there is no statistical difference between the age bracket 16-20 years and 31-35 years (P=0.747).

Table 3 showed the distribution of *Salmonella* agglutinin titres in 110 male subjects. The findings showed that more male had *Salmonella* agglutinin titres for *Salmonella* Typhi O (81.8%) and *S. Typhi* H (72.7%). More so, most male had *Salmonella* agglutinin titres for *S. Paratyphi* C-O (56.4%), 50 (45.5%) had agglutinin for *S. Paratyphi* A-H.

The results in Table 3 also showed the number and percentage of sera with titres in 110 apparently male students. It was observed that only agglutinins for *S. Paratyphi* A-O, B-O, C-O, B-H and C-H were present in the sera of the subject up to the titre of 160 and at frequency ranging from 1.6-58.8%. Remarkably, agglutinins for *S. Typhi* O and H were not present in the sera of male students. Also, the percentage of male subjects with titre 1:80 is significantly higher than those with titre 1:160 (P=0.001).

Table 4 showed the distribution of *Salmonella* agglutinin titre in 90 female students. The results showed that more females had *Salmonella* agglutinin titres for *S. Typhi* H (44.4%), while the least agglutinin titre for female students was for *S. Typhi* O (7.8%).

The results in Table 4 also showed the number and percentage of sera with end titres in 90 apparently healthy female students. It was observed that only agglutinins for *S. Paratyphi* A-O, C-H and *S. typhi* O and H were present in the sera of the subjects up to the titre of 160 and at frequencies ranging from 2.5% - 25.0%. Moreover, the percentage of female subjects with titre 1:160 is significantly lower than those with titre 1:80 (P=0.001).

Table 1: Prevalence of Widal positive sera (*Salmonella* agglutinin titre) based on gender

Sex	No. of sera tested (%)	No. of Widal positive (%) ^a	No. of Widal negative (%) ^a
Male	110 (55.0)	90 (81.8) ^b	20 (18.2)
Female	90 (45.0)	30 (33.3) ^c	60 (66.7)
Total	200 (100)	120 (60.0)	80 (40)

*a = not significantly different (P=0.221). *b and c =significantly different (P=0.028)

Table 2: Prevalence of Widal positive sera (*Salmonella* agglutinin titre) based of age group

Age group	No. of sample tested	Positive titre (%) ($\geq 1:80$)	Negative titre (%)(< 1:80)
16-20	30	16 (53.3)	14 (46.7)
21-25	80	57 (71.3)	23 (28.7)
26-30	56	31 (55.4)	35 (44.6)
31-35	29	14 (48.3)	15 (51.7)
36-40	5	2 (40.0)	3 (60.0)

Table 3: Number (%) and distribution of sera with agglutinin end titre among male students

<i>Salmonella</i> antigen	No. of sera tested	No. (%) of Widal positive	Titre < 1:80	Titre 1:80 ^a	Titre 1: 160 ^b
<i>S. Paratyphi</i> A-O	110	31 (28.2)	11 (35.5)	10 (32.3)	10 (32.3)
<i>S. Paratyphi</i> B-O	110	30 (27.3)	4 (13.3)	20 (66.1)	6 (20.0)
<i>S. Paratyphi</i> C-O	110	62 (56.4)	19 (30.6)	40 (64.5)	3 (4.8)
<i>S. Typhi</i> O	110	90 (81.8)	5 (5.5)	85 (94.4)	0
<i>S. Paratyphi</i> A-H	110	50 (45.5)	15 (30.0)	35 (70.0)	0
<i>S. Paratyphi</i> B-H	110	34 (30.9)	6 (17.6)	8 (23.5)	20 (58.8)
<i>S. Paratyphi</i> C-H	110	40 (36.4)	11 (27.5)	29 (72.5)	0
<i>S. Typhi</i> H	110	80 (72.7)	10 (22.5)	70 (87.5)	0

*a and b = significantly different (P=0.001).

148 **Table 4:** Number (%) and distribution of sera with agglutinin end titre among female students

Salmonella antigen	No. of sera tested	No. (%) of widal positive	Titre < 1:80	Titre 1:80 ^a	Titre 1: 160 ^b
<i>S. Paratyphi</i> A-O	90	20 (22.2)	5 (25.0)	10 (50.0)	5 (25.0)
<i>S. Paratyphi</i> B-O	90	5 (5.6)	2 (40.0)	3 (60.0)	0
<i>S. Paratyphi</i> C-O	90	6 (6.7)	2 (33.3)	4 (66.7)	0
<i>S. Typhi</i> O	90	7 (7.8)	2 (28.6)	3 (43.0)	2 (28.6)
<i>S. Paratyphi</i> A-H	90	23 (25.6)	6 (26.1)	17 (73.9)	0
<i>S. Paratyphi</i> B-H	90	30 (33.3)	20 (66.7)	10 (33.3)	0
<i>S. Paratyphi</i> C-H	90	29 (32.2)	5 (17.2)	20 (68.9)	4 (13.8)
<i>S. Typhi</i> H	90	40 (44.4)	15 (38.0)	24 (60.0)	1 (2.5)

149 * a and b = significantly different (P =0.001)

150 DISCUSSION

151 In this study, 120 (60%) of the 200 (100%) blood samples gave positive Widal reaction. This
152 indicates a high prevalence of typhoid fever in the sampled population. Similarly, high
153 prevalence of positive Widal reaction greater than 60% was also reported in Nigeria [5, 10,
154 11]. The possible explanations for the high prevalence of *Salmonella* Typhi and Paratyphi
155 titres among apparently healthy individuals may be that the bacilli are persistent in the human
156 host at sub-clinical levels [12]. It may also be due to the repeated subclinical infections with
157 either of *Escherichia*, *Shigella*, *Citrobacter* or *Proteus* species which shared common 'O' or
158 'H' antigens with *Salmonella* spp [13, 14, 15]. Alternatively, other individuals may have
159 developed tolerance to frequent exposure of small inocula of *Salmonella* Typhi and Paratyphi
160 [12] which may lead to high rate of asymptomatic infections. However, most of the subjects
161 may not be having the active diseases. This is in agreement with previous observation [16].
162 Lower prevalence of positive Widal reactions has been reported in Nigeria, other African
163 countries and Asia. These includes 24.4% in Owerri, Nigeria, [17] 41.2% in Borno, Nigeria,
164 [18] 21.3% and 22% prevalence rate in Kenya and Ethiopia respectively, [13, 19] 50%
165 prevalence rate in Nepal [8] and 31.1%, 12.1%, 27.3% have been reported in India [20, 21,
166 22]

167 The Widal test reaction involves the use of bacterial suspensions of *S. Typhi* and *S. Paratyphi*
168 A, B and C, treated to retain only the 'O' and 'H' antigens. The antigens suspensions are

employed to detect corresponding immunoglobulins in the serum of a patient suspected of having typhoid fever. During the primary immune response in acute typhoid fever, IgM somatic O immunoglobulin appears first, while the IgG flagella H immunoglobulin appear much more later, but persists for longer period in circulation [7, 11].

The age distribution pattern of Widal positive test showed that the age group 21-25 years (57 cases) had the highest positive result while the least was found in the age bracket 36-40 years (5 cases). This may be due to the fact that majority of subjects in the study area are within that age population. This result was found to contradict previous findings in which it was reported that 26-35 years and 5-15 years had the highest and lowest Widal positive results respectively [17]. However, the findings of this study is in agreement with previous reports which suggested that prevalence of typhoid fever in endemic areas is considered high among school-age children and young adults. Older adults are presumably relatively resistant due to frequent boosting of immunity [23].

Also, in this study, sera from males were more Widal positive than females. This is in agreement with previous studies [11, 16, 24]. This is probably as a reflection of different eating habits and level of personal hygiene [11]. Contrary to our findings however, higher prevalence rate among female than male have been reported [17, 20]. According to them, this may be due to the fact that females are more vulnerable to such disease due to poor health conditions and environmental factors associated to these women.

Among the 110 males tested, the titre of Salmonella 'O' were higher than those of 'H', where as in 90 females, Salmonella 'H' titres were higher than those of 'O'. This is in agreement with the report of Okonko *et al.* [11] but differ with the report of Ibekwe *et al.* [5].

A negative agglutination test may be for one of several reasons which include absence of infection by *S. Typhi*, the carrier state, an inadequate inoculum of bacterial antigen in the host to induce antibody production, technical difficulty or errors in the performance of the test, previous antibiotic treatment and variability in the preparation of commercial antigens.

Apparently healthy refers to the absence of disease based on clinical signs and symptoms normally assessed by routine laboratory procedures and physical examinations. The subjects involved in this study are apparently healthy and are most likely unaware of their health conditions and therefore are likely to infect others. This is because transmission of typhoid fever is reported to be chiefly among apparently healthy carriers especially among those who work as food handlers [25]. Many of them become asymptomatic carriers after an acute typhoid episode.

The etiologic agent of typhoid can establish a chronic asymptomatic infection of the human gall bladder by forming a biofilm. On reaching the gall bladder, the bacterium can induce active local infection or exist asymptotically in a chronic carrier state [26, 27]. The chronic typhoid carrier state can occur following symptomatic or subclinical infections of *S. typhi*. Among untreated cases, 10% usually shed bacteria for 3 months after initial onset of symptoms and 2-5% becomes chronic carriers. Bacteria shed by asymptomatic carriers contaminate food and water and account for much of the person-to-person transmission of serovar Typhi in developing countries [26, 27, 28].

Typhoid and paratyphoid fevers are common in developing countries, basically due to the problem of unsafe drinking water, inadequate sewage disposal and flooding, [11] poor hygiene practice, indiscriminate and open defecation due to lack of toilet facilities, etc. Health education about personal hygiene, provision of safe water supply, proper sanitation system, excluding disease carriers from food handling are among the critical public health measures that can be taken to prevent typhoid and paratyphoid [11]. Although bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. Consequently, a rapid, accurate and sensitive test such as the one we have reported may be useful to differentiate typhoidal from non-typhoidal febrile illnesses in a typhoid endemic region.

CONCLUSION

The findings of this study established that *Salmonella* agglutinins are common among apparently healthy individuals with variable Widal agglutination titre. Although many studies have highlighted the limitations of Widal test for laboratory diagnosis of typhoid fever, however, the test is still of diagnostic value in endemic areas provided the results are interpreted with utmost degree of care and accuracy.

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