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Seroprevalence of *Salmonella typhi and Salmonella paratyphi* among the First Year Students of University of Ilorin, Ilorin - Nigeria

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Abstract: *Salmonella typhi* and *Salmonella paratyphi*, the causative agents of typhoid fever and paratyphoid fevers, are found in individuals with the disease, but can also be found in people without the disease and who do not show any symptoms of being ill. This work reports the prevalence and incidence of *Salmonella typhi* and Salmonella paratyphi in apparently healthy first year students of the University of Ilorin, Ilorin-Nigeria. Two hundred students [115 (57.5%) males and 85 (42.5%) females] were recruited for the study after informed consent. Blood samples were collected from the students and examined for the presence and the levels of *Salmonella typhi* and *Salmonella paratyphi* antibodies by Widal agglutination technique. The standard *Salmonella* 'O' and 'H' suspension (ANTEC diagnostic products) were used as antigens. In this study, the titre of 1:160 is considered significant. Higher significant titre of antibody was detected for *Salmonella typhi* while significant titre was not detected for paratyphi B and C. The result was also used to relate to the source of drinking water. Highest percentage (39.6%) of significant titre of antibodies to *Salmonella* was detected among subjects who drink sachet water while the least (26.6%) was detected among subjects who drink treated water. Gender distribution also showed that higher percentage (42.4%) of females had significant titre of antibodies to *Salmonella* as against 33% observed among the males. Treatment of water before drinking is therefore advocated.

Key words: Salmonella typhi · Salmonella paratyphi · Antibodies · Asymptomatic · University of Ilorin

INTRODUCTION

Typhoid fever otherwise known as enteric fever is a common worldwide illness, transmitted by the ingestion of food or water contaminated with feces from an infected person. Typhoid remains a serious health problem in many regions of the world, with some 16 million cases and 600 000 deaths occurring annually [1]. The annual incidence of typhoid is estimated to be about 17 million cases worldwide [2]. At the beginning of the 19th century, typhoid was defined on the basis of clinical signs, symptoms and pathological (anatomical) changes. The term "enteric fever" is a collective term that refers to typhoid and paratyphoid [3]. Paratyphoid fevers are usually milder than typhoid fever [4] although not without danger. The milder form of the disease, paratyphoid fever, is caused by serovars paratyphi A, B and C *Salmonella enterica* subspecies *enteric* [5]. The name "typhoid" was given by Louis in 1829, as a derivative from typhus, the impact of this disease falls sharply with the application of modern sanitation techniques. Nigeria, like many other tropical and developing countries, has been described as an endemic zone for typhoid fever [6].

Of the over 2500 Salmonella serotypes recognized to date [7], about 1400 infected humans and other warm-blooded vertebrates, which are classified into Salmonella subgroup I now often referred to as Salmonella enterica subspecies enteric [8-9]. Although most of these Salmonella serotypes cause self-limiting gastroenteritis in humans, four cause typhoid fever, including S. typhi and S. paratyphi A, B and C [10]. Salmonella paratyphi C is a member of serogroup C1 [11]. It is known to cause typhoid in humans. Unlike S. typhi, it is also known to

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occasionally infect animals [12]. In several ways, S. paratyphi C is similar to S. typhi, including the possession of a large pathogenicity island, SPI7, with genes coding for the Vi (virulence) antigen and other genes potentially associated with virulence [13-14].

Flying insects feeding on feces may occasionally transfer the bacteria through poor hygiene habits and public sanitation conditions, some of the carriers may be out working as food handlers, processors in food industries or water industries and even household workers who take care of daily feeding. Typhoid and paratyphoid germs are passed in the faeces and urine of infected people. Infection of people occurs following ingestion of food or drinking beverages that have been handled by a person infected or by drinking water that has been contaminated by sewage containing the bacteria. Once the bacteria enter the person's body they multiply and spread from the intestines, through the bloodstream [2]. Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria. These people are consider a source of infection for others. The transmission of typhoid and paratyphoid in less-industrialized countries may be due to contaminated food or water. In some countries, shellfish which was taken from sewage-contaminated beds is an important route of infection. Where water quality is high and chlorinated water piped into the house is widely available, transmission is more likely to occur via food contaminated by carriers handling food [2]. Infection through contaminated surgical equipment and person-to-person contact in hospital has also been reported [15]. In endemic areas, identified risk factors for the disease include eating food prepared outside the home (e.g., ice cream, flavoured iced drinks) by street vendors [16], drinking contaminated water [2, 17], poor housing with inadequate facilities for personal hygiene [18].

In Nigeria, enteric fevers caused by *S. typhi* and *S. paratyphi* are not only endemic [6, 19] but constitute a great socio-medical problem [20], being responsible for many cases of pyrexia of unknown origin [21], high morbidity and mortality [22-24]. Therefore, the aim of this study is to determine the prevalence of the different species of Salmonella (thereby highlighting the health implication) in the apparently healthy newly admitted students of the University of Ilorin and determine the potential of the different sources of drinking water in the Ilorin metropolis to spread the pathogen.

MATERIALS AND METHOD

Study Population: After due permission from the Hospital management and informed consent was obtained, 200 samples of blood was collected from first year students of the University of Ilorin for 2009/2010 academic session who are undergoing medical examination at the university health services center. The subjects comprise of 115 males and 85 females (age range: 16-35 years).

Sample Collection and Processing: Five milliliters of venous blood was collected from each of the subjects into sterile bottles and was allowed to clot. The serum was then pipetted into sterile ependorf tubes and stored at -20°C for further analysis. The collected subjects' variables are age, gender and source of drinking water. The test and interpretation of the results were done according to the guidelines of the kit's manufacturer.

Widal Agglutination Test: The test was carried out using ANTEC febrile antigen kit (United Kingdom). The rapid slide screening test was first carried out, followed by the tube agglutination test according to the manufacturer's specifications. All the positive results were subjected to the tube agglutination test.

RESULTS

The number of people showing different titres of plasma antibody to *S. typhi* and *S. paratyphi* 'O' and 'H' antigens obtained from the sera of 200 (100%) first year students of the university of Ilorin for the 2009/2010 academic session [115 (57.5%) males and 85 (42.5%) females] are shown in Table 1.

The significant and insignificant titres of antibodies in different sources of drinking water for *S. typhi* and *S. paratyphi* 'O' and 'H' antigens are shown in Tables 2 and 3 respectively. Table 2 shows that 28 (28.0%) of those who drink tap water had significant titre of antibodies (≥1:160) to *S. typhi* and *S. paratyphi* 'O' antigens. Among those who drink bore hole water, 12 (37.5%) had significant titre of antibodies to *S. typhi* and *S. paratyphi* 'O' antigens, 18 (34.0%) of those who drink sachet water, 4 (26.7%) of those who drink treated water had significant titre of antibodies to *S. typhi* and *S. paratyphi* 'O' antigens as shown in Table 2.

Table 1: Titre of plasma antibodies to *S. typhi* and *S. paratyphi* 'O' and 'H' antigens

	Number of individuals with:							
	S. typhi		*S. paratyphi A*		*S. paratyphi B*		*S. paratyphi C*	
Titre	O	H	O	H	O	H	O	H
1:20	56	64	182	123	168	192	191	192
1:40	28	58	13	45	28	7	8	8
1:80	53	47	3	29	4	1	1	0
1:160	63	31	0	3	0	0	0	0
Total	200	200	200	200	200	200	200	200

Table 2: Prevalence of significant and insignificant titre in different sources of drinking water for *S. typhi* and *S. paratyphi* 'O' antigens

Source of drinking water	Number with: Significant titre (%)	Insignificant titre (%)	Total
Tap water	28 (28.0)	72 (72.0)	100
Bore hole	12 (37.5)	20 (62.5)	32
Sachet water	18 (34.0)	35 (66.0)	53
Treated water	04 (26.7)	11 (73.3)	15
Total	62 (31.0)	138 (69.0)	200

Table 3: Prevalence of significant and insignificant titre in different sources of drinking water for *S. typhi* and *S. paratyphi* "H" antigens

Source of drinking water	Number with: Significant Titer (%)	Insignificant Titer (%)	Total
Tap water	18 (18.0)	82 (82.0)	100
Bore hole	03 (09.4)	29 (90.6)	32
Sachet water	10 (18.9)	43 (81.1)	53
Treated water	01(06.7)	14 (93.3)	15
Total	32 (16.0)	168 (84.0)	200

Table 4: Percentage significance in drinking water source

Source of drinking water	Total sample collected	Number (%) Significance
Tap	100	37 (37.0)
Bore hole	32	12 (37.5)
Sachet water	53	21 (39.6)
Treated water	15	04 (26.6)
Total	200	74 (37.0)

Table 3 shows that 18 (18.0%) of those who drink tap water had significant titre of antibodies to *S. typhi* and *S. paratyphi* 'H' antigens. Among those who drink bore hole water, 3 (9.4%) had significant titre of antibodies to *S. typhi* and *S. paratyphi* 'H' antigens, 10 (18.9%) of those who drink sachet water, 1 (6.7%) of those who drink treated water had significant titre of antibodies to *S. typhi* and *S. paratyphi* 'H' antigens as shown in Table 3.

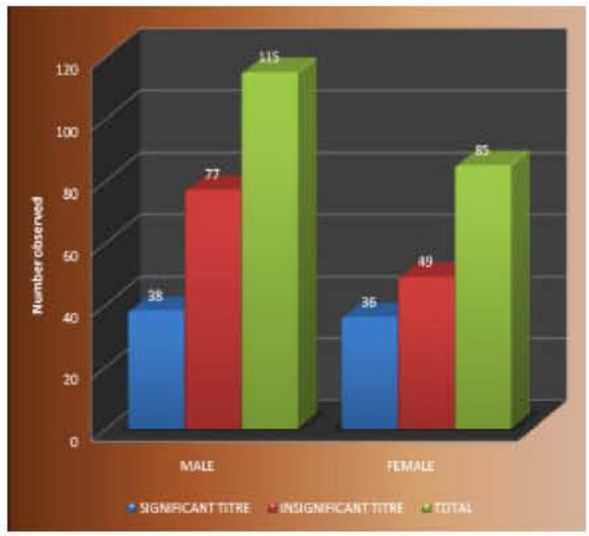

Fig. 1: Significant and insignificant titre in male and female

Out of the 53 samples tested for subjects who drink sachet water, 21 had significant antibody titre giving the highest percentage of 39.6%. This is followed by bore hole with 37.5% (12 out of 32) and tap water with 37.0% (37 out of 100) while the least was observed among subjects who drink treated water with 26.6% (4 out of 15) as shown in Table 4.

Figure 1 shows the significant and insignificant titre in male and female. Out of the 115 samples tested for males, 38 (33.0%) showed significant titer while in the females, 36 out of 85 (42.4%) showed significant titre (Figure 1)

DISCUSSION

In this study, all the 200 (100%) blood samples collected from the apparently healthy first year students of the University of Ilorin tested showed agglutination reaction at different dilutions. However, the titre of 1:160 is considered significant. All the subjects which were tested in this study are apparently healthy thereby supporting the earlier observation by Outi *et al.* [25] and Adeleke *et al.* [26] that widal reaction is more relevant in diagnosing post-infection complications when *S. typhi* may not be isolated.The highest prevalence (31.5%) of significant titer of antibodies was observed for *S. typhi* group 'O' antigen followed by *S. typhi* group 'H' antigen (15.5%). The prevalence of 1.5% was also observed for *S. paratyphi* group 'H'. Antibodies to groups 'O' and 'H' antigens of *S. paratyphi* B and C as well as group 'O' antigens of *S. paratyphi* A were not detected in this study. The *S. paratyphi* C as a typhoid agent [27], is not reported as frequently as *S. typhi* or *S. paratyphi* A or B, partly because this pathogen shares the same antigenic formula with *S. choleraesuis* and *S. typhisuis* and clinical identification of *Salmonella* isolates usually does not go beyond serotyping, although molecular methods are available to reliably distinguish *S. paratyphi* C from other Group C members [28]. The occurrence of paratyphoid serotype was not associated with domestic water source probably because paratyphoid infections are more commonly transmitted through flies and contaminated foods, typhoid on the other hand is transmitted mainly through contaminated water [29]. This study revealed high significant titre of antibodies to *Salmonella* in subjects whose source of drinking water were; sachet water (39.6%), bore hole (37.5%) and tap water (37.0%). Lower antibody titre (26.6%) was however observed for subjects who drink treated water. The common methods of treating water among households in Nigeria include; boiling, chlorination, addition of sodium aluminate ("alum"), addition of "water guard" (which contains 1.0% sodium hypochlorite).

Typhoid fever and paratyphoid are associated with poor environmental conditions, especially in poor countries [30]. The reason for the observation of low amount of significant titre among people who drink treated water may not be farfetched since the water is relatively free of microbes as a result of the treatment. On the other hand, most of the sachet water, commonly known as 'pure' water in Nigeria, are not registered by the National Agency for Food, Drug Administration and Control (NAFDAC) and are prone to faking. Standard procedures are usually not followed in the production of these sachet waters. The source of the water can vary widely, the personal hygiene of the manufacturers may be questionable and also the motive of the manufacture which in most cases is profit making is also worrisome. Tap water directed into homes is checked by National primary drinking water regulation, which protects drinking water by limiting the level of specific contaminants. However, most of the pipes conveying this water to different homes have leakages along the way thereby leading to contamination of the water by soil microorganisms including *Salmonella*. Most of the subjects who claim to drink water from bore holes actually drink water from shallow wells as most of the so called bore holes did not meet the required specification. This explains the reason for the relatively high antibody titre detected among subjects who drink water from these sources thereby underscoring the need for proper treatment of water before drinking no matter the source. The distribution pattern of the infections seems uncertain in Nigeria and appears to show geographical variation. Some studies found that enteric fevers are more prevalent in males than in females [30-33], but Zailani *et al.* [20] found no influence of age, sex and social class on the distribution pattern of *S. typhi/paratyphi* in Ile-Ife, south western Nigeria.

Our study, however, showed that more females (42.0%) had significant antibody titre than their male counterparts (33.0%) with significant antibody titre although there is no statistical significance ($p>0.05$). It should also be noted that all the subjects tested in this study are assymptomatic thereby raising doubt on the reliability of some clinicians on serological tests for diagnosis of typhoid fever. The probability of infection and the disease is generally lower when an individual is exposed to small numbers of pathogen. This is because there must be a certain minimum number of cells of the pathogen in the body to produce enough damage to cause disease symptoms [34]. Small doses of pathogens often result in a higher percentage of asymptomatic infections [34]. Although these healthy people showed no symptoms of typhoid fever but had significant titre, probability is that they do not have enough bacteria in them to overcome their immune system and cause an illness, hence they are apparently healthy but have *Salmonella typhi* in their system.

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