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Prevalence of Typhoidal *Salmonella* Infections and Associated Risk factors in Kaduna Metropolis, Nigeria

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ABSTRACT

Introduction: *Salmonella* species *Salmonella typhi* and *Salmonella paratyphi*, types A, B, and C are the causative agents of enteric fever. This disease continues to pose a severe threat to public health, especially in developing countries. This study investigated the prevalence of typhoidal *Salmonella* infections and the associated risk factors in Kaduna Metropolis, Nigeria. **Methods**: A questionnaire was used to obtain information from 250 patients attending four selected government hospitals and 50 healthy individuals as the control. Blood samples from participants were obtained to determine the blood groups and genotypes, and stool specimens were used to isolate typhoidal *Salmonella* species. **Results**: Thirty cases were among symptomatic patients and two among controls showing a total prevalence of 10.6%. Among the 32 isolates, 25 were *S. typhi* (78.1%), and seven were *S. paratyphi* A (21.9%), revealing an approximate ratio of 4:1. Factors significantly associated with the infection prevalence included age, source of drinking water, and frequency of infection. Blood groups and genotypes were not statistically associated with the infection; however, individuals with blood group O and genotype *AA* were more commonly infected. Also, females, the age group 20-29, singles, and unemployed, showed more infections. **Conclusion**: The prevalence, the ratio of typhoidal *Salmonella* species, and the associated risk factors call for public health and control measures, including the provision of suitable drinking water and improving living and sanitary conditions.

INTRODUCTION

Enteric fever is a collective term for typhoid and paratyphoid infections caused by *Salmonella typhi* and *Salmonella paratyphi* A, B, and C [1]. *Salmonella paratyphi* A is the most prevalent type, while types B and C are rarely reported [2]. Typhoidal *Salmonella* bacteria are Gram-negative bacilli transmitted via the fecal-oral route; people acquire infections via ingesting contaminated food or water [1]. These enteric bacteria are restricted to humans [3] and have obtained the ability to overcome mucosal barriers and spread systemically even in immune-competent individuals via the acquisition of virulent factors absent in non-typhoidal *Salmonella* serotypes [4]. The most important reservoirs of the infection are short-term convalescents or chronic human carriers [5]. If left untreated, enteric fever may result in shock, organ failure, or even death [6].

Evidence gathered so far through genomics and clinical investigations have provided insight into host restriction and pathogenesis of typhoidal *Salmonella* infections. Research has shown that there might be a possible association between individuals' genetic factors and susceptibility to typhoid and paratyphoid infections [7]. Vaccination, commonly proven efficient in controlling other infectious diseases, is effective against typhoid fever only alongside public health enlightenment [8].

According to the Centre for Disease Control and Prevention (CDC), chlorination of drinking water has dramatically decreased typhoid fever transmission in developed countries like the United States [8]. However, the story is different in developing countries like Nigeria, where people live in poor sanitary conditions and have no access to appropriate health care and suitable drinking water [9]. Genetic components such as blood grouping, Rhesus factor (*Rh*), and genotypes seem to contribute to the susceptibility and outcome of infectious diseases like typhoid and paratyphoid infections [7, 10].

Enteric fever continues to challenge clinicians, public health scientists, and food safety officials [9]. Therefore, understanding individuals' genetic compositions and associated risk factors will increase our knowledge to prevent and control typhoidal infection in developing countries. This study investigated the prevalence of typhoidal *Salmonella* infections and the associated risk factors in Nigeria.

MATERIALS AND METHODS

Study Area and Population. This cross-sectional descriptive study was conducted in Kaduna metropolis (Fig. 1), Kaduna State, Nigeria, from July 2018 to February 2019. The study population included 250 patients suspected of enteric fever based on clinical diagnosis and 50 healthy volunteers. The patients from 57 residential areas in Kaduna metropolis (30 in Kaduna North and 27 in Kaduna South) referred to four selected hospitals located in different parts of the metropolis, including General Hospital Sabon Tasha (GHST) representing Kaduna South-east, Gwamna Awan General Hospital (GAGH) Kakuri, Kaduna South-west, Barau Dikko Teaching Hospital (BDTH) Ungwan-Rimi and Yusuf Dantosho Memorial Hospital (YDMH) Tudun Wada, representing Kaduna North-east and west respectively.

Clinical samples. The blood samples were collected from all participants in bottles containing ethylenediaminetetraacetic acid (EDTA), and stool samples in clean, dry screw-top containers. The samples were transported to the laboratory (NNPC Industrial Clinic Laboratory, Kaduna).

Ethical approval was obtained from the Ministry of Health and Human Resources, Kaduna State (MOH/ADM/744/VOL.1/483), and the Ethical Committee of Barau Dikko Teaching Hospital Health Research, Kaduna State (Reference Number 18-0034). Written consent was obtained from all participants or their guardians (for the participants ≤ 15 years) enrolled in the study.

Isolation of typhoidal *Salmonella*. Typhoidal *Salmonella* bacteria were isolated using standard bacteriological procedures. A pinch of stool was enriched in Selenite F broth (HiMedia Laboratories Pvt. Ltd., India) and incubated at 37ºC for 6-12 h [14]. A loop full of the enriched broth was cultured by streaking onto the *Salmonella-Shigella* Agar plates (Lab M Limited, Heywood Lancashire, United Kingdom). The plates were incubated aerobically at 37ºC for 18-24 h and then examined for characteristic typhoidal *Salmonella* colonies, i.e., smooth, colorless, or transparent, and flat colonies with a clear halo or black center [14-16]. The identity of the isolates was confirmed as *S. typhi* or *S. paratyphi* A by biochemical tests including Gram staining, Kligler Iron Agar (KIA), and urease test (Titan Biotech Ltd., Rajasthan, India), motility, indole, and lysine decarboxylation or deamination, Methyl Red-Voges Proskauer (MR-VP) test, and citrate utilization test (HiMedia Laboratories Pvt. Ltd., India), catalase and oxidase tests [16-20] (Fig. 2).

Blood group and genotype. About 2-5 ml of blood was collected aseptically by vein puncture into EDTA bottles. Blood group and *Rh* factor were determined by mixing a drop of whole blood with anti-A, B, and D blood grouping reagents (Smart Diagnostics Kit, United Kingdom) for 45 s. Agglutination reaction was observed for blood groups and *Rh* reactivity as $AB^{+/-}$, $A^{+/-}$, $B^{+/-}$ and $O^{+/-}$. For genotype determination, gel electrophoresis was employed to separate DNA bands in blood samples using a Uniscope electrophoresis tank for about 15 min. The gels were stained with methylene blue 0.1%, and the migration patterns of DNA were used to identify the genotypes *AA*, *AS*, *SS*, and *AC* against known patterns.

Risk factors for typhoidal *Salmonella* infections. The investigated risk factors included sociodemographic data (gender, age, marital status, occupational status, type of family and level of education), genetic factors (blood group and genotype), living conditions (the type of toilet and residence, and the number of residents), and other predisposing factors (handwashing practice, traveling, the source of water for general use and drinking, source of food and frequency of infection). A well-structured questionnaire was used to collect sociodemographic data, clinical information, and predisposing factors.

Data Analysis. Data analyses were performed using IBM SPSS version 20 and Chi-square analysis. Results were presented as frequency tables, ratios, percentages, and bar charts. *P*-values less than or equal to 0.05 at 95% confidence level were considered significant for the parameters examined for the associations.

Clinical samples. Stool and blood samples were obtained from all participants. Blood samples were collected in bottles containing ethylenediaminetetraacetic acid (EDTA), and stool samples in clean, dry screw-top containers, respectively. The samples were immediately transported to the laboratory (NNPC Industrial Clinic Laboratory, Kaduna).

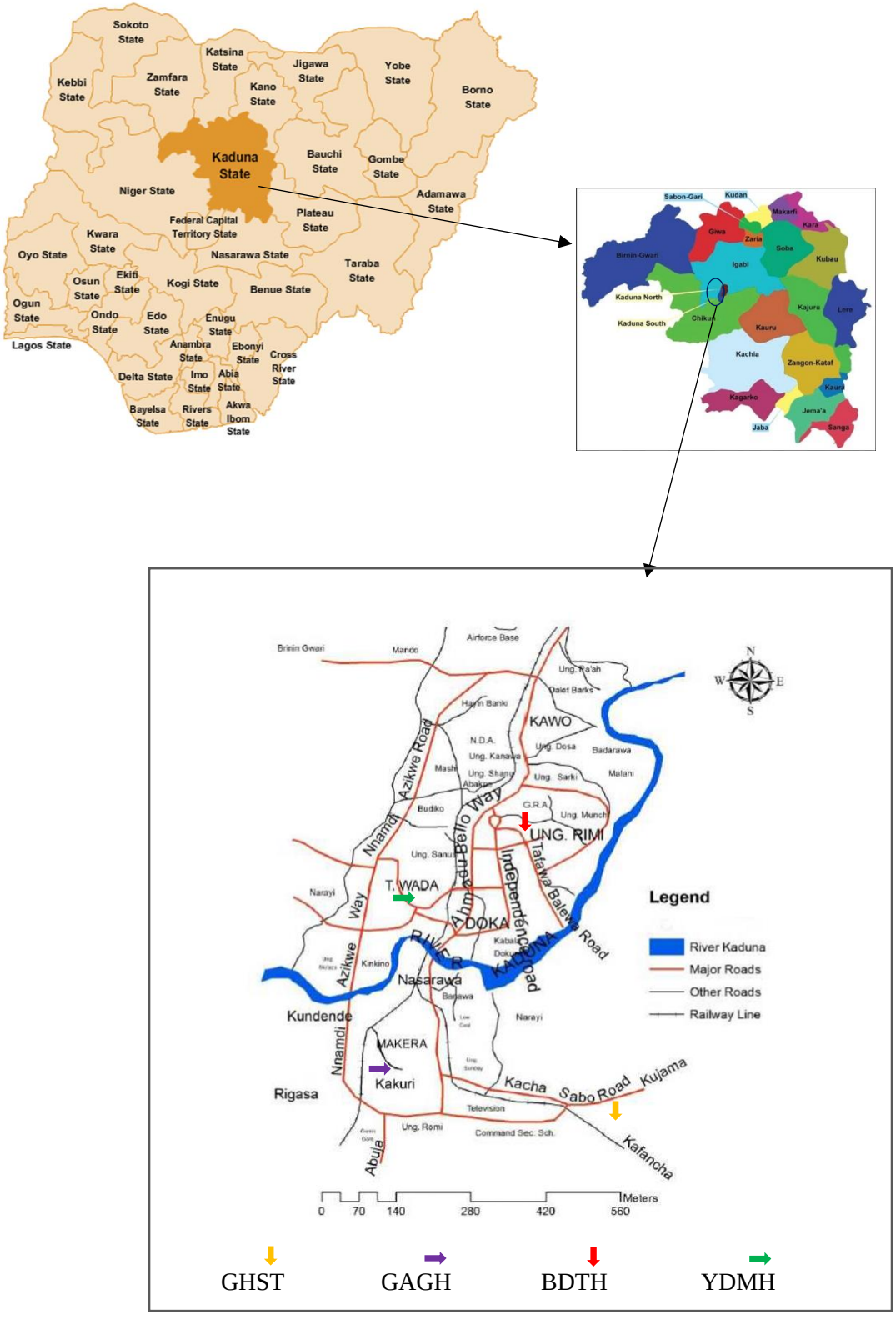

Fig 1. Map of Nigeria, Kaduna State, and the study area showing residential regions [11-13]

Isolation of typhoidal *Salmonella*. Typhoidal *Salmonella* bacteria were isolated using standard bacteriological procedures. A pinch of stool was enriched in Selenite F broth (HiMedia Laboratories Pvt. Ltd., India) and incubated at 37ºC for 6-12 h [14]. Using aseptic laboratory procedures, a loop full of the enriched broth was cultured by streaking onto the *Salmonella Shigella* Agar plates (Lab M Limited, Heywood Lancashire, United Kingdom). The plates were incubated aerobically at 37ºC for 18-24 h and then examined for characteristic typhoidal *Salmonella* colonies, i.e., smooth, colorless, or transparent, and flat colonies with a clear halo or black center [14-16]. The identity of the isolates was confirmed as either *S. typhi* or *S. paratyphi* A by biochemical tests including Gram staining, Kligler Iron Agar (KIA), and urease test (Titan Biotech Ltd., Rajasthan, India), motility, indole, and lysine decarboxylation or deamination, Methyl Red-Voges Proskauer (MR-VP) test, and citrate utilization test (HiMedia Laboratories Pvt. Ltd., India), catalase and oxidase tests [16-20] (Fig. 2).

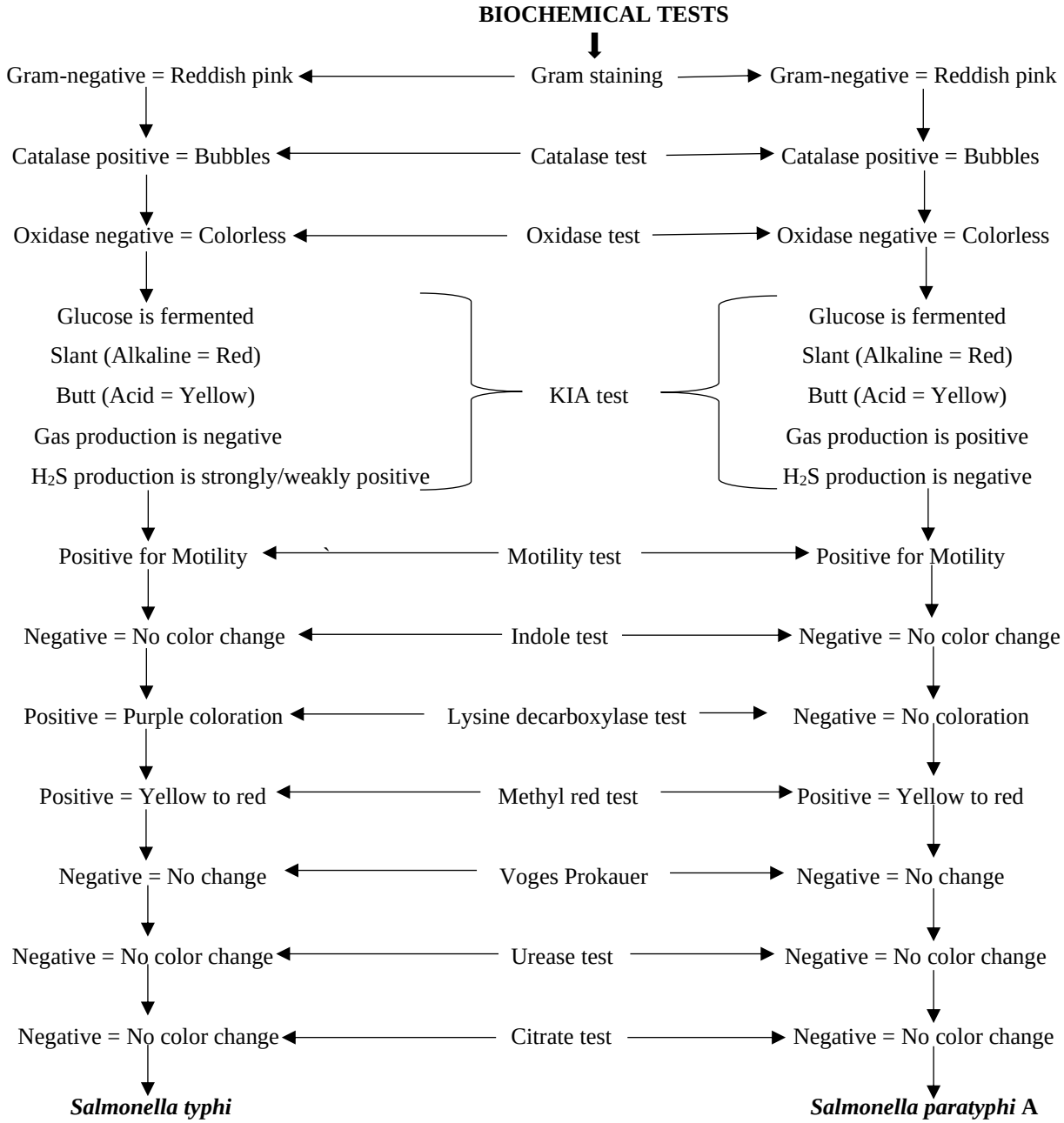

Fig. 2. Chart for identification of typhoidal *Salmonella* isolates, *S. typhi* or *S. paratyphi* A.

RESULTS

One hundred and sixty-three (163) males and 137 females participated in the study, revealing 15 and 17 cases, respectively (**Table 1**). Table 2 shows the participants' age group distribution and the number of cases in each group. The highest number of participants (97/300) was among the age group 20-29 years and showed the highest number of isolates (10/32) (**Table 2**). The least number of participants (3/300) were elderly (≥ 60 years), and none was positive.

Table 1. Typhoidal *Salmonella* infections among female and male participants.

Participants	Negative Individuals (No.)	*S. paratyphi* A (%)	*S. typhi* (%)	Typhoid infections (%)	Number of participants
Male	**148**	3 (9.4%)	12 (37.5%)	**15** (46.9%)	**163**
Female	**120**	4 (12.5%)	13 (40.6%)	**17** (53.1%)	**137**
Total	**268**	**7 (21.9%)**	**25 (78.1%)**	**32 (100%)**	**300**

Table 2. Typhoidal *Salmonella* infections among different age groups.

Age groups (Year)	Males (No.)	Females (No.)	Negative individuals (no.)	*S. paratyphi A* (%)	*S. typhi* (%)	Typhoid infections (%)	Number of Participants
≤ 9	27	17	**36**	2 (6.3%)	6 (18.7%)	**8** (25.0%)	**44**
10-19	19	27	**41**	1 (3.1%)	4 (12.5%)	**5** (15.6%)	**46**
20-29	50	47	**87**	2 (6.3%)	8 (25.0%)	**10** (31.3%)	**97**
30-39	37	29	**62**	0	4 (12.5%)	**4** (12.5%)	**66**
40-49	22	13	**33**	1 (3.1%)	1 (3.1%)	**2** (6.2%)	**35**
50-59	5	4	**6**	1 (3.1%)	2 (6.3%)	**3** (9.4%)	**9**
≥ 60	3	0	**3**	0	0	**0**	**3**
Total	163	137	**268**	7 (21.9%)	25 (78.1%)	**32 (100%)**	**300**

Overall, thirty-two *Salmonella* isolates were obtained in this study, showing a 10.6% prevalence (8.3% for *S. typhi* and 2.3% for *S. paratyphi*) (**Table 3**). Of the 32 *Salmonella* isolates obtained, 25 (78.1%) were *S. typhi*, and 7 (21.9 %) were *S. paratyphi* A, showing an approximate ratio of 4:1. Among the 250 patients with a clinical diagnosis, 30 had infections, 24 with *S. typhi*, and 6 with *S. paratyphi* A, while among individuals with no clinical manifestations (controls), two had infections with each of *S. typhi* and *S. paratyphi* A (**Table 3**). North Kaduna and South Kaduna had 11 and 21 cases, respectively (**Table 3**).

Among 32 cases, 22 (68.8%) were among singles (194/300), and 10 (31.2%) belonged to married individuals (106/300). Unemployed participants (162/300) had 20 cases (62.5%), while self-employed (69/300) and employed participants (69/300) had 7 and 5 cases (21.9% and 15.6%), respectively. Participants from nuclear families (252/300) had 25 of the 32 cases, while those from the extended (29/300) and polygamous (19/300) families had 5 and 2 cases, respectively. Eleven cases were from participants with a tertiary level of education (153/300), 11 from individuals with secondary education (65/300), nine from those with no education (51/300), and one from those with primary education (31/300).

Table 4 shows that the most common blood group among 300 participants was O^+ (58.3%). Other blood groups were B^+ (13.7%), A^+ (9.3%), O^- (8.3%) and AB^+ (7.7%). Among 32 cases, 21 belonged to individuals with blood group O, followed by the individuals in the blood groups B (8/32), AB (2/32), and A individuals (1/32) (Table 4). The highest infection rate was observed in the *AA* genotype (22/32), followed by *AS* genotype (10/32). Among the rest of the participants, seven had genotype *SS* and one genotype *AC*; none was positive for typhoidal infection (Table 4).

Our survey revealed that most of the infections (30/32) were among participants who used water cisterns (280/300), and the remaining two belonged to pit latrines (20/300). About 53.1% (17/32) of infection belonged to participants who lived in crowded habitats (129/300), and the remaining 15 (46.9%) were among those who lived in more comfortable situations (171/300). Similarly, 11 cases came from residences with ≤ 5 inhabitants (135/300), and 21 cases from those with > 5 inhabitants (165/300).

Handwashing practices were rated on a scale of 0 to 5, with 5 as the highest level. No infection was among the 9 participants who were in level 5. The majority of the cases (18/32) belonged to level 2 (156/300), followed by level 1 (35/300) with 6 cases. Four cases belonged to each of level 3 (64/300) and 4 (33/300). Participants who traveled frequently (182/300) had 20 of the 32 cases, followed by those who rarely traveled (61/300), showing

7 cases. Those with no history of travel (57/300) showed the remaining 5 cases.

Participants who used well water for general use (117/300) showed the highest cases (17/32), followed by borehole water users (115/300) that had 9 cases. The remaining 6 cases came from those who used pipe water alone or alongside either borehole or well water. Similarly, those who drank borehole water mainly alongside alternative sources showed 13 cases, while those who drank sachet water, well, and pipe-borne water as primary sources of drinking water alongside alternatives showed 8, 6, and 3 cases, respectively. The remaining 2 cases belonged to suckling babies who used none of the sources.

Table 3. Distribution of typhoidal *Salmonella* infections in different hospitals and residential areas

Category of participants	Negative individuals (no.)	*S. paratyphi* A (no.)	*S. typhi* (no.)	Total cases	Number of Participants
Patients in Hospitals					
BDTH	**44**	2	4	**6**	**50**
GAGH	**80**	2	11	**13**	**93**
GHST	**51**	1	5	**6**	**57**
YDMH	**45**	1	4	**5**	**50**
Total	**220**	6	24	**30**	**250**
Controls					
Healthy Volunteers	**48**	1	1	**2**	**50**
Residential areas					
Kaduna North	**87**	3	8	**11**	**98**
Kaduna South	**181**	4	17	**21**	**202**
Total	**268**	7	25	**32**	**300**
Percentage (%)	**89.3**	2.3	8.3	**10.6**	**100**

Barau Dikko Teaching Hospital (BDTH), Gwamna Awan General Hospital (GAGH), General Hospital Sabon Tasha (GHST), and Yusuf Dantosho Memorial Hospital (YDMH).

Many participants (171/300) who consumed homemade food alongside foods prepared outside homes showed the highest cases (15/32), followed by those who consumed only homemade food (88/300) with 13 cases. Participants who only consumed foods prepared outside of their homes (35/300) showed 2 cases. The remaining two cases were among the suckling babies (6/300) that consumed neither of the food sources. Clinical information showed that 85.4% of participants (256/300) had previously contracted typhoid fever. Among the remaining 14.6% (44/300) with no history of typhoid fever, four were positive, showing an incidence of 9.1% in this study. About 56.3% of cases (18/32) belonged to participants (160/300) with a history of vaccination against typhoid fever in childhood or adulthood. Also, most of the cases (28/32) were among participants (255/300) who experienced the infection at least once a year.

DISCUSSION

The present study investigated the prevalence of typhoidal *Salmonella* infections and associated risk factors in Kaduna metropolis, Nigeria. The prevalence rate of 10.6% obtained in Kaduna metropolis in this study was lower than 17.2% in Calabar and 24.4% in Owerri, Nigeria, using stool culture and Widal test respectively [21, 22]; 19% in Ethiopia, obtained from both blood culture and Widal test [23], and 62.7% obtained in Karu, Nigeria for *S. typhi* by Widal test and stool culture [24]. This study's low prevalence rate might be due to the methods used, seasonal variations, or other factors within the state and selected hospitals. The approximate infection 4:1 ratio of *S. typhi* to *S. paratyphi* A infection in this study agrees with data in India [2], indicating that *S. paratyphi* A cases have also increased in Nigeria. Interestingly, in our study, two cases were among healthy volunteers (n=50), showing asymptomatic carriers, suggesting the need to monitor these individuals' disease.

Table 4. Prevalence of typhoidal *Salmonella* infections and genetic factors, i.e., blood groups and genotypes

	Negative individuals (no.)	*S. paratyphi* A (no.)	*S. typhi* (no.)	Total No. of Isolates (%)	Number of Participants
Blood Group					
A-	4	-	-	-	4 (1.3%)
A+	27	1	-	1 (3.1%)	28 (9.3%)
AB-	4	-	-	-	4 (1.3%)
AB+	21	-	2	2 (6.3%)	23 (7.7%)
B-	1	-	-	-	1 (0.3%)
B+	33	2	6	8 (25.0%)	41 (13.7%)
O-	21	1	2	3 (9.4%)	24 (8.3%)
O+	157	3	15	18 (56.3%)	175 (58.3%)
Genotype					
AA	159	4	18	22 (68.8%)	181 (60.3%)
AS	101	3	7	10 (31.2%)	111 (37.0%)
AC	1	-	-	-	1 (0.3%)
SS	7	-	-	-	7 (2.3%)
Total	**268**	**7**	**25**	**32 (100%)**	**300 (100%)**

The risk factors found to be statistically associated with the infection were participants' age (p=0.034), source of drinking water (p=0.033), and frequency of infection (p=0.001). Typhoid fever might have a relation with blood phenotypes [25]. However, we found no statistical association between genotypes and blood groups with typhoidal infection in this study. In this study, like in South-east Nigeria, the blood group O comprised the most infected individuals [7].

Females showed a higher infection (53.1%) in this study, like women in Ogume, Owerri, and Karu in Nigeria [7, 22, 24]. Like the results obtained in Owerri, Nigeria, the highest infection rates were in the age group 20-29 (Table 2), showing this age group's vulnerability to enteric fever [22]. Eight of 32 cases (25%) were in the age group 0-9, showing agreement with a high incidence of typhoid fever observed in children ≤ 15 years of age [24, 26]. These results suggest that hygiene awareness, especially among those handling children, is necessary. The eating habits of the age group 20-29 might have contributed to the most cases, as there was a significant association between age group and source(s) of food (p=0.001). Contaminated foods and waters and unhygienic food preparation in homes seemed to have most likely exposed these age groups to the infection.

No significant association existed between marital status and typhoidal infection (p=0.054), but singles were more susceptible to infection. The difference might be related to the food source, as singles commonly eat foods prepared outside the home.

In Dakar, Bangladesh, typhoid fever showed a strong relationship with socioeconomic conditions [25]. However, in our study, similar to Ethiopia, no association was found between occupational (p=0.829) or educational status (p=0.149) and typhoidal infection [23], suggesting that occupational status and education level in developing countries have less effect on exposure to infection than personal hygiene.

In this study, no statistical association was between living conditions and typhoid infection. However, living in group households with poor hygiene showed to be a risk factor for enteric fever [27]. Also, a crowded living condition was strongly associated with typhoid fever [25]. Higher numbers of typhoidal *Salmonella* infections among individuals living in crowded habitats with shared toilets in this study suggested that such conditions may foster the disease. Therefore, improved living conditions, proper sewage disposal, and maintenance of sanitary conditions will no doubt limit the risk of infection.

Unlike a study in Ethiopia, where poor handwashing habit was significantly associated with typhoid infection, no statistical association existed between typhoidal infection and the level of handwashing (p=0.839) in this study [23]. However, no cases in the level 5 handwashing group suggested that proper practice of handwashing could be useful in preventing the disease.

No association existed between the infection and frequency of travel (p=0.991). However, frequent travelers included more cases, suggesting that traveling might increase infection rates via contaminated food and water and poor hygiene conditions.

There was a statistical relationship between drinking water sources and typhoid infections (p=0.033), similar to reports for Dhaka, Bangladesh, Sokoto, Nigeria, and Cameroon [25, 28, 29]. The majority of cases in this study (13/32) were borehole users, followed by sachet water (8/32) and well water (6/32), similar to the study in Cameroon, which showed that borehole or well water as the primary source of drinking water was significantly associated with typhoid fever [29]. In Niger, borehole users had the highest percentage of anti-*Salmonella* antibodies [30]. Another study in Singida, Tanzania, revealed that land drilled wells as the primary water

source with the proximity to pit latrines very likely resulted in contamination of water [31].

No association existed between vaccination and typhoidal infection (p=0.237). This finding shows that vaccination had no effect on typhoidal infection and corroborate an earlier report that vaccines alone could not eliminate typhoid fever, except when combined with the appropriate public health measures [8,32]. Nevertheless, vaccines should be available, especially in the case of outbreaks [33]. An association between the history of typhoid fever and frequency of infections (p=0.001) in this study revealed the recurring pattern of infection, which also shows that a first-time infection did not confer immunity to a subsequent infection [32].

The prevalence (10.6%), incidence (9.1%), and 4:1 ratio of typhoidal *Salmonella* infection by *Salmonella typhi* and *Salmonella paratyphi* A in this study necessitate intensified efforts for the control and prevention of the infection. The risk factors associated with the infection in the present study also call for public health interventions such as enlightenment of risk factors associated with the disease, provision of portable drinking water, improving living conditions, and ensuring sanitary measures.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest associated with this article.

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