Sumerianz Journal of Biotechnology, 2021, Vol. 4, No. 4, pp. 133-143
ISSN(e): 2617-3050, ISSN(p): 2617-3123
Website: https://www.sumerianz.com
DOI: https://doi.org/10.47752/sjb.44.133.143

Original Article Open Access

Clinico-Epidemiological Studies of Plasmodium Falciparum and Salmonella Typhi Co-Infection among Patients Attending Selected General Hospital in Northern Nigeria

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Article History
Received: August 3, 2021
Revised: October 7, 2021
Accepted: October 11, 2021
Published: October 15, 2021

Abstract

Study on the prevalence of co-infection between *Plasmodium falciparum* and *Salmonella typhi* among patients in Northern Nigeria was carried out. The study is cross-sectional designed to determine the socio-demographic characteristics as well as the risk factors for malaria and typhoid. A total of 100 consented patients of age group of 21-40 years were recruited for the study. A structured questionnaire was administered, and venous blood samples were collected and analyzed using standard microbiological methods. The isolated salmonella species were biochemically characterized, and subjected to antimicrobial susceptibility test using Kirby-Bauer disc diffusion method. The prevalence of malaria and typhoid was found to be 56% and 68% respectively. The prevalence of malarial parasite and *Salmonella typhi* infections was 40%. Females recorded low malarial infection of 56.9% compared to their male counterparts 43.1% (P= 0.510). The age group, educational levels and occupations of the study participants were not associated with the likelihood of having malarial parasite infection (P= 0.297, 0.15 and 0.503 respectively). Participants who did not sleep under the insecticide treated nets were more likely to have malaria than those who did ($P \leq 0.0001$). The educational levels of the study participants were statistically associated with *Salmonella typhi* infection (P= 0.026). Water sources, use of pit latrine, hand washing before and after meal were significantly associated with *Salmonella typhi* infections (P= <0.0001 and P= 0.003 respectively). The isolates of *Salmonella typhi* and *Salmonella paratyphi* were found to be sensitive to chloramphenicol (86.8%), ciprofloxacin (80.9%) and amoxicillin (79.4%), but relatively resistant to penicillin and augmentin that recorded sensitivities of 19.1% and 35.3% respectively. The prevalence of malaria and typhoid infections as well as malarial parasite and *Salmonella typhi* co-infections is high among the study population. Fortunately, the isolated bacteria are highly sensitive to chloramphenicol and ciprofloxacin.

Keywords: Antimicrobial; Co-infection; Malaria; Prevalence; Seroprevalence.

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1. Introduction

Malaria and *Salmonella typhi* infections are very common in the tropics especially in the Sub-Saharan African countries like Nigeria. Malaria-*Salmonella typhi* co-infection is also very common. Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans belonging to the *Plasmodium* sp [1]. It causes symptoms that typically include fever, fatigue, vomiting, and headaches. In life-threatening and severe cases it can cause jaundice, seizures, coma, or death. Malaria parasites belong to the genus *Plasmodium* (phylum *Apicomplexa*). In humans, malaria is caused by *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* and *Plasmodium knowlesi*. Among those infected, *Plasmodium falciparum* is the most common species identified in 75% followed by *P. vivax* 20% [2]. Although *Plasmodium falciparum* is the most prevalent malaria parasite on the African continent, it is responsible for most malaria-related deaths globally [3]. Recent evidence suggests that *Plasmodium vivax* malaria is also associated with potentially life-threatening conditions almost as much as that caused by *Plasmodium falciparum* infection [4]. Malaria is an acute febrile illness. In a non-immune individual, symptoms appear seven days or more (usually 10–15 days) after the infective mosquito bite. The early symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize as malaria. If not treated within 24 hours, *Plasmodium falciparum* malaria can progress to severe illness, often leading to death. Malaria and *Salmonella typhi* infections cause high morbidity and mortality in most countries in Sub-Saharan Africa, including Nigeria. This burden is further worsened by their co-infection. There are many methods of their diagnosis which can be used to measure their prevalence in a community to enable designing policies for treatment and prevention. According to the World Health Organization (WHO) estimates, released in December 2015, there were 214 million cases of malaria in 2015 and 438 000 deaths. Between the year 2000 and 2015, malaria incidence among populations at risk fell by 37% globally; during the same period, malaria mortality rates among populations at risk decreased by 60%. An estimated 6.2 million malaria deaths have been averted globally since 2001. It was reported that sub-Saharan Africa was home to 88% of malaria cases and 90% of malaria deaths [4]. Although malaria incidence among populations at risk has fallen by 37% globally and mortality decreased by 60%, 214 million malaria cases and 438, 000 deaths were recorded in 2015 Typhoid fever is still common in the developing world, where it affects about 21.5 million persons each year. Typhoid fever can be prevented and can usually be treated with antibiotics [5].

Typhoid fever is a life-threatening illness caused by the bacterium *Salmonella typhi.* In the United States, it is estimated that approximately 5,700 cases occur annually. Most cases (up to 75%) are acquired while traveling internationally. Typhoid fever is very common in the developing world where it affects about 21.5 million persons each year [6]. *Salmonellae* which are the etiologic agents of typhoid are gram-negative motile bacilli [4]. *Salmonella* species have been implicated in a spectrum of other diseases, including enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteremia, endovascular infections, focal infections (e.g. osteomyelitis), and *enterocolitis* typically *Salmonella typhimurium* and *Salmonella enteritidis* [7]. The transmission of salmonellae to a susceptible host usually occurs via consumption of contaminated foods. The most common sources of salmonellae include beef, poultry, and eggs. Almost any type of food product could serve as a source for infection, including peanut butter, as seen during a recent outbreak of more than 600 cases [8].

In the last decades, emergence of multidrug resistant salmonella species and changes in the epidemiology of typhoid fever has been reported by various researchers [9, 10]. Because Malaria and *Salmonella typhi* co-infection rate is high in most parts of poor developing nations, it was scantily documented in these environments. This research work focused on the prevalence of malarial parasite and *Salmonella typhi* as well as co-infection of *Salmonella typhi* and malarial parasite among the various group of patients attending the out-patient clinics of General Hospital in Northern Nigeria, with view to screen the patients for malaria and typhoid infections, and to determine the prevalence of *Plasmodium falciparum* and *Salmonella typhi* co-infection in the study population as well as to identify the risk and demographic factors that are associated with *Plasmodium falciparum* and *Salmonella typhi* infections. Also, to isolate, biochemically characterize and determine the antimicrobial susceptibility profile of the isolated salmonella species.

2. Materials and Methods

2.1. Sample Collection and Sample Processing

A total of 100 venous blood samples were collected from consented patients who presented with ≥ 4 days of fever attending General Hospital in Northern Nigeria, based on Helsinki Declaration Guidelines [11], and carefully dispensed into an EDTA anti coagulated container. The samples were transported to the laboratory immediately for processing. Prior to the collection of the samples, patient were given consent form and well-constructed questionnaires to fill under the researcher's guidance. Blood samples were centrifuged, and the serum was divided into aliquots and stored at the temperature of −20°C.

2.2. Rapid Diagnostic Test for Malaria

Using the micropipette, a drop of blood sample from the EDTA bottle was dropped on the test-end of the malaria Rapid Diagnostic Test (RDT) strip (EzDx™ Malaria (pf) rapid detection kit, Advy Chemical Pvt. Ltd, India). A drop of buffer was added to the drop of blood sample on the strip. It was allowed to stand for 10 to 20 minutes (Plate 1). The result was read and interpreted based on the appearance of the indicator lines that appeared on the immunochromatographic strip [12].

Plate-1. Cassettes of Rapid Diagnostic Test for the screening of malarial parasite

2.3. Widal Test for the Screening of Salmonella Typhi and Salmonella Paratyphi

On reaching the laboratory, a volume of 5ml of the collected blood sample was centrifuged at the speed of 1,500 rpm for 2 minutes to obtain the blood serum. Eight drops of the serum were dispensed in juxtaposition on the surface of sterile white tile, and a drop of each corresponding Widal kit (RAPID WIDAL, Bio Lab® Diagnostics Private Limited, India), and anti-sera was added onto the placed serum. The preparation was manually rocked for 1 minute, after which the result was read and recorded by comparing it with the titer value for antigen O and antigen H of *Salmonella typhi* and *Salmonella paratyphi* respectively. Caution was taken while manually rocking the preparation on the tile so that the different samples were not mixed up [13].

2.4. Blood Culture for Isolation of Salmonella

A quantity of 5ml of the whole blood was added to blood culture medium (Thioglycollate broth) and incubated at 37°C for 7 days with periodic subculture of the broth in blood agar to monitor any visible appearance of colonies. Each discrete colony was sub-cultured into Salmonella-Shigella agar to obtain pure cultures. Suspected colonies were smeared on slides, Gram stained and biochemically characterized using standard microbiological methods such as catalase, oxidase, indole, urease, lactose, motility, citrate, Voges Proskauer and Klinger Iron Agar utilization tests [14].

2.5. Antimicrobial Susceptibility Test of Salmonella Typhi and *Salmonella paratyphi* Isolates

Antimicrobial susceptibility trend of the isolated *Salmonella typhi* and *S. paratyphi* was carried out using Kirby-Bauer disk diffusion method as adopted by Umar, *et al.* [15]. This was by transferring the pure isolates into sterile normal saline to obtain bacterial density of 3×10^8 organism per milliliter McFarland standard. The culture was streaked uniformly on Muller Hinton agar, and discs of antimicrobials were mounted on the surface of the streaked inoculum. The plates were incubated at 37°C for 24 hours. Each of the cultures was examined for zone of growth inhibition using micrometer. The following antimicrobial agents (size of zone of inhibition) were used: amoxicillin (≥ 17 mm), tetracycline (≥ 15 mm), chloramphenicol (≥ 18 mm), streptomycin (≥ 15 mm), penicillin (≥ 15 mm), ciprofloxacin (≤ 31 mm), Trimethoprim-Sulfamethoxazole (≥ 16 mm), augmentin (≥ 18 mm), and gentamicin (≥15 mm) [16, 17].

2.6. Ethical Considerations and Data Analysis

The data obtained was sorted out and entered for analysis into the Statistical Package for Social Sciences (SPSS) IBM® Inc. version 20.0. Descriptive and inferential statistics was used to analyze the dependent variables (presence of malaria or typhoid infection and absence malaria and typhoid infection) and independent variables which included the risk factors and demographic factors for malaria and typhoid infections. The work was conducted following ethical approval by the Health Research and Ethic Committee of the Kaduna State Ministry of Health. Permission was also sought for and granted by the Head of Department of Laboratory Services, General Hospital, Zaria, Kaduna State, Nigeria. Written consent was obtained from the participants and confidentiality was ensured by serializing the participants and making them anonymous. Data collected remained undisclosed to the public. Data entered into electronic devices were pass-coded and only individuals that are directly involved in the study were allowed access.

3. Results

3.1. Socio-Demographic Characteristics of the Study Participants

Majority of the study participants were females who constituted 51.0% of the study participants. Most of the study participants were within the age group of 21-40 years of age. This was followed by those within the age group of 1-20 years of age and constituted 45.0%. The study participants have some level of education as most of them have even reached tertiary level and these constituted 40.0%. Most of the study participants were students who constituted 64.0%. Other participants were farmers and this constituted 17.0% (Table 1).

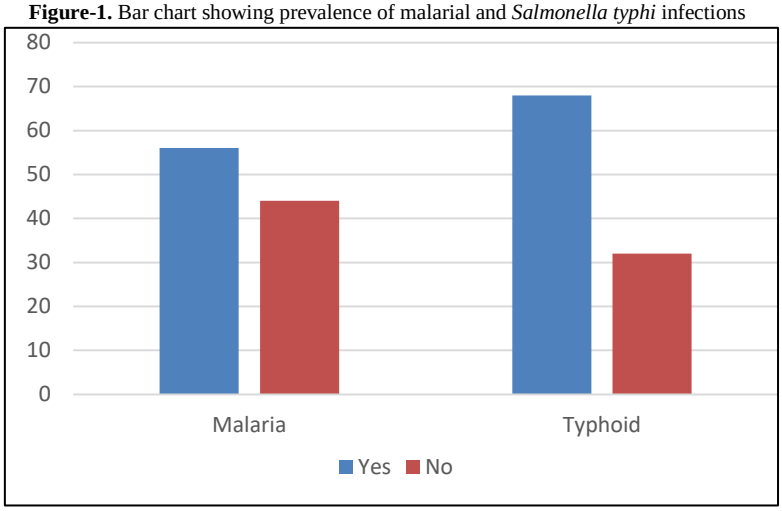
Table-1. Socio-demographic characteristics of the study participants (N = 100)

Variables	Number	Percentage prevalence
Gender		
Male	49	49.0
Female	51	51.0
Age group (years)		
1-20	45	45.0
21-40	31	31.0
>40	24	24.0
Educational level		
Primary	18	18.0
Secondary	35	35.0
Tertiary	40	40.0
Traditional/Religious	7	7.0
Occupation		
Farming	4	4.0
Student	64	64.0
Civil servant	17	17.0
Trading	15	15.0

3.2. Seroprevalence of Malarial Parasite and *Salmonella Typhi* Among the Study Population

The prevalence of malaria parasites among the participants was 56%. The Seroprevalence of *Salmonella typhi* was 68% (Figure 1).

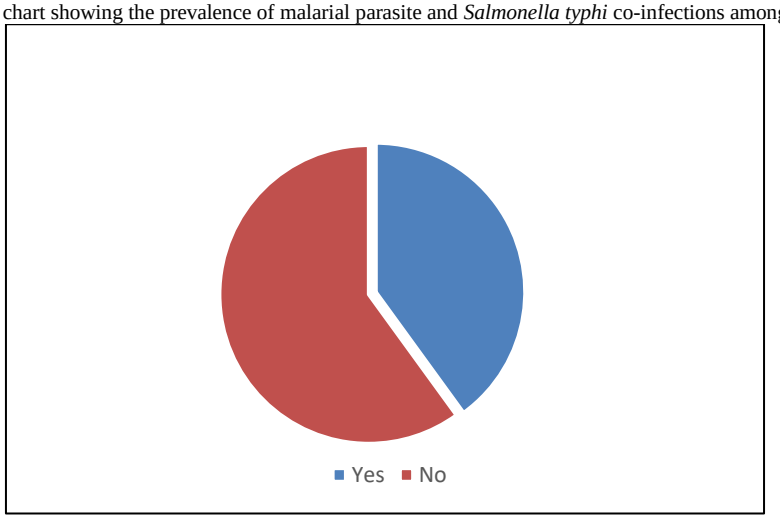
Figure-1. Bar chart showing prevalence of malarial and *Salmonella typhi* infections



3.3. The Prevalence of Malarial Parasite and *Salmonella typhi* co-Infection Among the Study Participants

The prevalence of malarial parasite and *Salmonella typhi* co-infections was 40% which constituted 40 participants (Figure 2).

Figure-2. Pie chart showing the prevalence of malarial parasite and *Salmonella typhi* co-infections among the patients



3.4. Risk and Demographic Factors Associated with Malarial Parasite Infection Among the Study Participants

The female participants constituted 51 out of which 29 (56.9%) had malarial infection. The females were not as likely to have malarial parasites as their male counter-parts. This was however not statistically significant (*P*= 0.510). The age group, educational levels and occupations of the study participants were not associated with the likelihood of having malarial parasite infection. These were not statistically significant with p-values of 0.297, 0.15, and 0.503 respectively. Out of the total 55 participants that slept under the insecticide treated nets, 22 (40%) had malarial infection. But 34 (75.6%) participants that did not sleep under the insecticide treated nets had malarial infection. Participants that did not sleep under the insecticide treated nets were more likely to have malaria than those who did. This was statistically significant with χ^2 test value of 12.698 and a p value less than 0.0001. It was also found that participants who had nets on their doors and windows at their houses, had no stagnant water around their houses, had not had blood transfusion and had malaria chemoprophylaxis were less likely to have malarial parasite infections. These were statistically significant with p-values <0.0001, <0.0001, 0.037 and 0.045 respectively. It was however found that the use of insect repellent, having bushes around the house, travelling to malaria endemic areas as well as having sickle cell anaemia were not associated with having malarial parasite infection. These were statistically significant with p-values of 0.171, 0.230, 0.226, 0.168 and 0.797 respectively (Table 2).

Table-2. Risk and demographic factors associated with malarial parasite infection among the study participants. (N = 100)

Variables	Presence of malaria				Total	df	χ^2	Critical value
	Positive		Negative					
	n	%	n	%				
Gender						1	0.031	0.031
Male	27	55.1	22	44.9	49			
Female	29	56.9	22	43.1	51			
Age group (years)						2	2.427	0.297
1-20	29	63.0	17	37.0	46			
21-40	15	45.5	18	54.5	33			
>40	12	57.1	9	42.9	21			
Educational level						3	10.399	10.399
Primary	16	88.9	2	11.1	18			
Secondary	19	54.3	16	45.7	35			
Tertiary	18	45.0	22	55.0	40			
Traditional/Religious	3	42.9	4	57.1	7			
Occupation						3	2.352	0.503
Farming	2	50.0	2	50.0	4			
Student	37	57.8	27	42.2	64			
Civil servant	7	41.2	10	58.8	17			
Trading	10	66.7	5	33.3	15			
Sleeping under ITN						1	12.698	0.000*
Yes	22	40.0	33	60.0	55			
No	34	75.6	11	24.4	45			
Nets on doors and windows						1	14.152	0.000*
Yes	17	36.2	30	63.8	47			
No	39	73.6	14	26.4	53			
Using insect repellent						1	1.873	0.171
Yes	32	50.8	31	49.2	63			
No	24	64.9	13	35.1	37			
Stagnant water around house						1	20.698	0.000*
Yes	31	86.1	5	13.9	36			
No	25	39.1	39	60.9	64			
Bushes around the house						1	5.180	0.23
Yes	17	77.3	5	22.7	22			
No	39	50.0	39	50.0	78			
Travelled to malarious area						1	1.469	0.226
Yes	13	68.4	6	31.6	19			
No	43	53.1	38	46.9	81			
Have Sickle cell anaemia						1	1.904	0.168
Yes	7	77.8	2	22.2	9			
No	49	53.8	42	46.2	91			
Had Blood transfusion						1	4.342	0.037*
Yes	8	88.9	1	11.1	9			
No	48	52.7	43	47.3	91			

Had malaria chemoprophylaxis						1	4.008	0.045*
Yes	1	16.7	5	83.3	6			
No	55	58.5	39	41.5	93			
Exposed body in the evenings						1	0.066	0.797
Yes	10	58.8	7	41.2	17			
No	45	55.4	37	44.6	83			

Key: * = statistically significant; ITN = Insecticide treated net

3.5. Socio-Demographic Factors Associated with Malarial and Salmonella Typhi Co-Infections Among the Study Participants

Fifty one participants were female and out of these, 32 (62.7%) had *Salmonella typhi* infection. Of the 49 total male participants, 36 (73.5%) had *Salmonella typhi* infection. The gender of the participants was not associated with the likelihood of having *Salmonella typhi* infection. This was not statistically significant with a p value of 0.250 (χ^2 = 1.321). The educational levels of the study participants were statistically significantly associated with *Salmonella typhi* infection with p-value of 0.026 (χ^2 = 0.024). The age groups and occupations of the study participants were not statistically significantly associated with *Salmonella typhi* infection with p-value 0.274 and 0.489 respectively.

Out of the 33 participants that use well as their source of drinking water, 31 (93.9%) had *Salmonella typhi* infection while only two of them did not. Twenty nine (74.4%) participants that used borehole also had high rate of *Salmonella typhi* infection. Only few participants that used tap and river as their source of drinking water had *Salmonella typhi* infection. The use of well and borehole was statistically significantly associated with *Salmonella typhi* infection with p-values <0.0001 each (χ^2 = 30.968 and χ^2 = 26.763 respectively).

Hand washing before and after meal were statistically significantly associated with *Salmonella typhi* infection with p-values <0.0001 and 0.003 respectively. The use of pit latrine was associated with *Salmonella typhi* infection. This was statistically significant with a p-value <0.0001 (χ^2 = 28.217 at 2 df).

The habit of finger nail biting and having typhoid vaccination was not associated with *Salmonella typhi* infection. These were statistically significant with p-values <0.0001 and 0.003 respectively (χ^2 = 16.534 and 9.063 respectively at 1 df each) (Table 3).

Table-3. Socio-demographic and risk factors associated with *Salmonella typhi* and *Salmonella paratyphi* infections among the study participants. (N = 100)

Variables	**Presence of typhoid and paratyphoid infection**				**Total**	**df**	χ^2	**Critical value**
	Positive		Negative					
	n	%	n	%				
Gender						1	1.321	0.250
Male	36	73.5	13	26.5	49			
Female	32	62.7	19	37.3	51			
Age group (years)						2	2.591	0.274
1-20	33	71.7	13	28.3	46			
21-40	19	57.6	14	45.4	33			
>40	16	76.2	5	23.8	21			
Educational level						3	9.246	0.026*
Primary	17	94.4	1	5.6	18			
Secondary	20	57.1	15	42.9	35			
Tertiary	25	62.5	15	37.5	40			
Traditional/Religious	6	85.7	1	14.3	7			
Occupation						3	2.425	0.489
Farming	4	100.0	0	0.0	4			
Student	44	68.0	20	31.2	64			
Civil servant	11	64.0	6	35.3	17			
Trading	9	60.0	6	40.0	15			
Source of drinking water						3	30.968	0.000*
Well	31	93.9	2	6.1	33			
Borehole	29	74.4	10	25.6	39			
Tap water	7	28.0	18	72.0	25			
River	1	33.3	2	66.7	3			
Hand washing before meal						2	26.763	0.000*
Never	1	100.0	0	0.00	1			
Sometimes	43	93.5	3	6.5	46			
Always	24	45.3	29	54.7	53			
Hand washing after meal						2	28.590	0.000*

Never	4	100	0	0	4			
Sometimes	39	95.1	2	4.9	41			
Always	25	45.5	30	54.5	55			
Type of toilet used								
Bush/open space	1	100	0	0.0	1	2	28.271	0.000*
Pit latrines	55	85.9	9	14.1	64			
Water closet	12	34.3	23	65.7	35			
Habit of nail biting						1	16.534	0.000*
Yes	26	100.0	0	0.0	26			
No	42	56.8	32	43.2	74			
Typhoid vaccination						1	9.063	0.003*
Yes	20	95.2	1	4.8	21			
No	48	60.8	31	39.2	79			
Eating from food vendors						2	58.893	0.000*
Never	4	13.3	26	86.7	30			
Sometimes	63	91.3	6	8.7	69			
Always	1	100.0	0	0.0	1			
Number of persons eating together						1	7.417	0.006*
Alone	44	60.3	29	39.7	73			
2-3 people	24	88.9	3	11.1	27			
Eating unwashed fruits/vegetables						1	1.961	0.161
Yes	4	100.0	0	0.0	4			
No	64	66.7	32	33.3	64			
Contact with typhoid patient						1	1.983	0.159
Yes	8	88.9	1	11.1	9			
No	60	65.9	31	34.1	91			

Key: * = Statistically significant

3.6. Biochemical Characterization of *Salmonella Typhi* and *Salmonella Paratyphi* Isolates

Preliminary microscopic analysis and biochemical characterization of the isolates were used for the identification of the isolates up to their species level. The findings of biochemical characteristics of the isolates were compared with that of the known taxa of salmonellae as reported in Bergey's Manual of Determinative Bacteriology [18] (Table 4).

Table-4. Biochemical characterization of the salmonella isolates

Microscopy	Lac	Cat	Ind	Oxid	Urea	Cit	Mot	V.P	KIA				Inference
									Slant	Butt	H_2S	Gas	
Gram-negative rod	−	+	−	−	−	−	+	−	Alkali	Acid	+	−	*Salmonella typhi*
Gram-negative rod	−	+	−	−	−	−	+	−	Acid	Acid	−	+	*Salmonella paratyphi*
Gram-negative, non sporing rod	−	+	−	−	−	+	+	−	Alkali	Acid	−	+	Other Salmonellae serotypes

Key: Lac= lactose, Cat= catalase, Ind= indole, Oxid= oxidase, Urea= urease, Cit= citrate, Mot= motility, VP= Voges Proskauer, KIA= Klinger iron agar, − = negative, + = positive

3.7. Antimicrobial Susceptibility Profile of *Salmonella Typhi* and *Salmonella Paratyphi* Isolates

The antimicrobial susceptibility pattern of the isolated *Salmonella typhi* and *Salmonella paratyphi* showed that the isolates were highly sensitive to chloramphenicol (86.3%), tetracycline (82.4%), ciprofloxacin (80.8%) and amoxicillin (79.4%). But, relatively resistant to penicillin and augmentin that recorded sensitivities of 19.1% and 35.3% respectively (Table 5).

Table-5. Antimicrobial susceptibility profile of *Salmonella typhi* and *Salmonella paratyphi* isolates

Antimicrobial	Potency (µg)	Sensitive	Resistance
Amoxicillin	10	54 (79.4%)	14 (20.6%)
Tetracycline	30	56 (82.4%)	12 (17.6%)
Chloramphenicol	30	59 (86.8%)	9 (13.2%)
Streptomycin	10	41 (60.3%)	27 (39.8%)
Ciprofloxacin	5	55 (80.9%)	13 (19.1%)
Trimethoprim-Sulfamethoxazole	1.25/23.75	40 (58.8)	28 (41.2%)
Augmentin	20	24 (35.3%)	44 (64.7%)
Gentamicin	10	39 (57.4%)	29 (42.6%)
Penicillin	10 U	13 (19.1%)	55 (80.9%)

4. Discussions

4.1. Prevalence of Malarial Parasite Infection Among the Study Participants

The prevalence of malarial parasite among the participants in this study was 56%. This finding is similar to findings of Ukaegbu and others who reported a prevalence of 54% among febrile patients in Jos, Nigeria [13]. It however contradicts the findings of Millicent who reported a prevalence of malaria to be 35.7% among patients attending a General Hospital in Makarfi, Kaduna state, Nigeria [19]. This prevalence is lower than that of the current study probably because microscopy was used in the latter study rather than rapid test used in this study. The rapid diagnostic test for malaria has higher false positivity than the microscopy [20]. The prevalence is however lower than the 71.4% reported by Udoh *et al* in Calabar in Cross River state (South south), Nigeria [21], but significantly higher than the 14.7% reported in Ibeshe community of Ikorodu, Lagos state (Southwest) Nigeria [22]. The large difference between the current study and that by Udoh *et al*. could probably be because Udoh's study was conducted during the raining season, rather than in the dry season when malaria transmission is much higher. However, a study carried out in the 6 geopolitical zones of the country in 2010, concluded that the prevalence of malaria is higher in the Southern part of the country than the Northern parts [23].

4.2. Seroprevalence of *Salmonella typhi* Infection among the Study Population

The seroprevalence of *Salmonella typhi* infection in this study was 68%. This finding did not agree with the prevalence of 18% reported by Esohe in Ikare, Ondo State, Nigeria [24]. The difference could be because the population in this study lived in typhoid endemic area [20] due to probably contaminated foods and drinking water. A high prevalence of typhoid infection is also not unexpected because of the high sensitivity of Widal test kit for the diagnosis of typhoid rather than relying on clinical acumen or on blood culture. The kits are also cheap and accessible with high accuracy and specificity [25]. Furthermore, the high prevalence of typhoid infection in both studies is attributed to lack of clean and potable drinking water and poor sewage disposal system in the study area [13].

4.3. Prevalence of Malaria and *Salmonella typhi* Co-infection Among the Study Population

The prevalence of malarial parasite and *Salmonella typhi* co-infections in this study was 40%. The finding agrees with the prevalence of 42% of malaria-typhoid co-infection reported by Okaegbu in Jos, Nigeria [13]. Although the findings are similar, in Ukaegbu's study, both Widal test and stool culture were done. This could have increased the yield and result in higher prevalence in the latter study. The finding in the current study is higher than the prevalence of 28% reported by Orok, *et al.* [26]. Even though the prevalence of malaria and typhoid co-infections was high in the current study, it should be noted that typhoid fever could cross-react with malaria using Widal test reagent [27] and lead to over diagnosis of typhoid fever especially in areas where there is high prevalence of malaria and typhoid infections [26]. This disagrees with the findings of Sumer, *et al.* [28] who reported low salmonella co-infection and related its mechanism with impaired mobilization of granulocytes through the heme and heme oxygenase which are released from hemoglobin due to breakdown of erythrocytes during malaria infection. However, Chowdhury, *et al.* [29] opined that the actual and precise underlying mechanism to explain the association between malaria and *Salmonella* associated infection is still unclear, although it seems clear that malaria predisposes to bacteremia and sepsis due to salmonella species.

4.4. Risk Factors and Demographic Factors Associated with Malaria

In this study, sleeping under an insecticide treated net, having net screens on the doors and windows, not having stagnant water around the house, having blood transfusion and malaria chemoprophylaxis were identified as factors that reduce the incidence of malaria among the study population. This finding agrees with the [30] report of behavioral factors that are associated with malarial parasite infection. These behavioral factors include agricultural activities including animal husbandry, having stagnant dirty water around living areas and none use of housing and bed nets that would protect people from exposure to mosquitoes due to lack of education and poor attitudes towards it. Although other known risk factors for malaria such as the use of insect repellants, having bushes around the house, being a sickle cell anaemia patient and exposing the skin in late evenings were not found to be statistically significantly associated with malarial infection, many studies have confirmed the existence of a positive relationship of the above factors with malarial infection [31].

4.5. Risk Factors and Demographic Factors Associated with *Salmonella typhi* Infections

More males (73.5%) were found to be more infected with typhoid infection than females (62.7%). This agrees with the findings of Ashraf and co-workers in Dhaka, Bangladesh that the incidence of typhoid infection is more in the males than in the females. This gender preponderance might be the reflection of health-care seeking behavior in Bangladesh which is largely controlled by cultural beliefs such as religion and patriarchy [32]. The participants majorly use well as their main source of water and 93.9% of these participants had typhoid fever. It was a similar finding by Farooq in Indonesia. The study presented the link between contaminated well water with the outbreak of typhoid fever. The wells are contaminated because most of them are sited very close to sewage disposal systems such as pit latrines [33].

Most of the participants that used pit latrine (85.9%) had typhoid infection. It was a similar finding by Malisa in Tanzania who reported that about 90% of those infected with *Salmonella* specie used pit latrines majorly against the water closet system [34]. Most of the study participants who only sometimes washed their hands before meal (93.5%) and after meal (95.1%) had typhoid infection. It was a similar report by the World Health Organization in 2001. Contaminated hands with *Salmonella* bacteria lead to direct inoculation of these organisms into humans [35].

4.6. Antimicrobial Susceptibility Pattern of the Isolated *Salmonella typhi* and *Salmonella paratyphi*

The antimicrobial susceptibility pattern of the 68 isolates of *Salmonella typhi* and *Salmonella paratyphi* recorded highest susceptibility to chloramphenicol 59 (86.8%), tetracycline 56 (82.4%), ciprofloxacin 55 (80.9%) and amoxicillin 54 (79.4%), with relative resistant to penicillin 13 (19.1%) and augmentin 24 (35.3%). This may be due to the ability of salmonellae to resist beta-lactam antibacterial agents by virtue of beta-lactamase enzyme secretion. Cavalier, *et al.* [36], documented that Gram-negative bacteria may become resistant to beta-lactam antibiotics by developing permeability barriers. This usually is caused by altered porin channels in the outer membrane that no longer allow the entrance and passage of antibiotic molecules into the cell. When beta-lactams cannot reach the Penicillin Binding Proteins (PBPs), the cell becomes resistant to penicillin and penicillin derivatives.

The susceptibility to chloramphenicol and ciprofloxacin conforms with the work of Singhal, *et al.* [37] who reported 95% susceptibility of *Salmonella typhi* to chloramphenicol and stable resistance to ciprofloxacin. However, the moderate resistance found in streptomycin and gentamicin may probably be as a result of genetic mutation of *Salmonella typhi* as a result of blind treatment and frequent self-medication by the typhoid patients especially at the chronic stage of the disease. This corroborates well with the studies of Humphries and Fang [38] who reported that a single mutation in chromosomal *gyr* A (encoding for subunit of DNA gyrase) located in quinolone resistance determining region (QRDR) confer high resistance to fluoroquinolones by *Salmonella typhi*. Ranveendran, *et al.* [39] added that nowadays *Salmonella typhi* developed resistance to some of the first line drugs of choice such as ciprofloxacin, and this is an indicative of the effects of indiscriminate use of fluoroquinolones. However, chloramphenicol must be used with caution due to its side effects on bone marrow especially in patients with type I hypersensitivity penicillin allergy [40].

5. Conclusion

Prevalence of malaria and typhoid infections was found to be high among the study population. Use of insecticide treated nets, having net screens on the doors and windows, having good drainages to avoid water stagnation around houses were found to be associated with malaria. The use of malaria chemoprophylaxis was found to be associated with lower incidence of malaria. Educational levels of the study participants were found to be associated with typhoid infection. Drinking contaminated well water and not washing the hands before and after meal and defaecation were associated with typhoid infection. Having uncut finger nails, thumb sucking habits, not having typhoid vaccination as well as eating together in large numbers were associated with typhoid infection. *Salmonella typhi* and *Salmonella paratyphi* were isolated and identified using standard biochemical methods. The isolates were found to be multidrug resistant, but they are found to be sensitive to various antimicrobials. Chloramphenicol showed promising results and proved to be a first line drug of choice for the treatment of typhoid, paratyphoid and non-typhoid fevers.

In this study, a high prevalence of malarial parasites, typhoid and their co-infections were found. There is therefore the need to reduce this prevalence which will go a long way to reducing the burden of these diseases. Widespread sensitization on the importance of sleeping under insecticide treated nets including providing it free or subsidizing it for the populace is imperatively important. Malaria chemoprophylaxis should be ensured in vulnerable groups such as the pregnant women and sickle cell anaemia patients. Also, increasing the literacy level of the populace will go a long way in reducing the spread of malaria and typhoid infections. Moreover, the antimicrobial susceptibility trend of *Salmonella typhi* and *S. paratyphi* should be determined periodically in order to monitor emergence of resistance to drugs routinely used in the treatment of typhoid and paratyphoid fevers.

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