Serological Screening for Malaria and Typhoid Fever in Febrile Patients Attending National Hospital, Abuja.

Bukola-Ajide¹*, Kemi-Ojegbile¹, Lillian-Adogo¹ and Philip-Alexander¹

¹Department of Biological Science, Bingham University, Karu, Nigeria

+2348138844356

ABSTRACT
A total of 300 blood samples were collected from patients for widal test into plain tubes and allowed to coagulate to get the serum. Blood samples for malaria investigation were collected in EDTA tubes, thick blood films were prepared and stained using field stain A and B and the Care Start Malaria HRP2 (Pf) was used for rapid qualitative detection of malaria. Overall, 40.67% of the total population were positive for malaria infection, 50% were positive for widal serological agglutination test for typhoid infection, the result indicates 27.33% co infection with malaria and typhoid fever among the patients. Salmonella species causing typhoid fever appear to be the most prevalent cause of febrile infection among the patients. Therefore, efforts should be made to find, more accurate, clinical and cultural methods of identifying and isolating Salmonella species in blood samples. In addition, proper laboratory tests should be carried out on samples collected from patients before the administration of drugs so as to avoid drug resistance.

INTRODUCTION
Malaria is primarily a vector-borne parasitic disease caused by Plasmodium species and transmitted to man through the bites of infected Anopheles mosquito, the most serious form of the disease is caused by Plasmodium falciparum while the other species (P. vivax, P. ovale, and P. malariae) induce milder disease in humans. However, the fifth species, P. knowlesi is a zoonosis that cause malaria in monkeys, but can also infect humans [17]. Blood transfusion is also believed to be a dreaded vehicle for the transmission of many parasitic diseases including malaria [7].

Typhoid fever (enteric fever) on the other hand is a systemic prolonged febrile illness caused by certain Salmonella serotypes. Salmonella enterica serotype typhi (S. typhi) and Salmonella enteric serotype paratyphi (S. paratyphi A, S. paratyphi B, and S. paratyphi C) are species that cause typhoid fever. S. typhi is the most common serotype of salmonella that causes typhoid fever [14, 15]. The estimated total number of world typhoid fever episode in 2010 was 13.5 million [4]. There are approximately 12.5 million estimated cases of typhoid fever each year, with more than 62% of these occurring in Asia and 35% in Africa [9]. Poor disposal of human excreta, poorly equipped latrine with water facility, poor hand washing habit, and untreated water usage are the main cause of transmission of typhoid fever in developing countries [10].

However, an association between malaria and enteric fever was first described in the medical literature in the 19th century and was named typhomalarial fever by the United States Army [19]. The relationship between the two diseases has been substantiated by studies from Africa, India and elsewhere [8]. Although malaria and typhoid are caused by very different and distinct organisms, a protozoan and a gram-negative bacillus and transmitted via different mechanisms, both diseases share rather similar symptoms. Thus it is very common to see patients undergoing both typhoid and malaria treatments even when their diagnosis has not been confirmed [12].

Like many other developing countries, accurate diagnosis of infectious diseases remains a challenge due to lack of skilled man-power and equipment making clinical diagnosis a common practice. It is therefore very important that clinical diagnosis be informed by appropriate epidemiologic data. Accurate diagnosis of a disease needs to be followed by appropriate treatment. However, because of drug resistance, it is usually necessary to carry out sensitivity tests before making an informed choice of an antibiotic for treatment. The purpose of this study is therefore to generate updated baseline data on co-infection of malaria and typhoid fever for clinic-epidemiologic purposes which will enhance better management and control, in addition to trying to understand the implications of these co-infections in disease severity by serologically screening febrile patients attending the National Hospital Abuja – Nigeria for malaria and typhoid fever by determining the co-infection of malaria and typhoid fever and to detect the cause(s) of fever when patients come down with febrile symptoms.

MATERIALS AND METHODS
Study Area and Design
This study was conducted from January to March 2016 at the National Hospital which is situated at plot 132, National Hospital road, P.M.B 425, Central Business District, Abuja, Nigeria and lies between latitude 9°0’ North and longitude 7°46’ East, with an elevation of 536m. Questionnaires were given to the febrile patients, to obtain their demographic information and their identity was kept confidential throughout this study.
Study Population
The study population consisted of 300 febrile patients made up of male and female of different ages and who were attending the National Hospital Abuja.

Ethical Approval
Ethical approval for this study was obtained from the ethics and research committee of the National Hospital Abuja.

Sample Collection
Blood collection and analysis were made using a technique of disinfection as described by Cheesbrough (2006). A standard and careful laboratory procedure was adopted in collecting the blood samples from the febrile patients.

Procedure for Widal test
Blood from 300 patients for Widal test was collected into plain bottles and allowed to coagulate; the tubes with the samples were centrifuged to separate the serum. Freshly collected serum was stored at 5 – 8°C in case of a delay in testing. Widal test was performed using Wondfo Widal slide test 8 × 5 ml (slide agglutination) and using the manufacturer’s instructions.

Procedure for Malaria test
Blood samples from 300 patients for Malaria investigation were collected into EDTA tube. Thick blood films were prepared from each sample. The thick films were stained in field stains A and B. The stained slide was allowed to air dry and was examined microscopically for malaria parasites (Ochei, et al., 2007). Also the CareStart Malaria HRP2 (Pf) was used for the rapid qualitative detection of malaria HRP2 (histidine-rich protein2) (P. falciparum) in the blood.

Statistical Analysis
The result was presented in a statistical method using the Chi-Square test. This is because it involves computing a test statistics as compared with the probability distribution of the standard printed in tables. Chi-Square is variously referred to as a test of homogeneity, randomness, association and independence.

RESULTS
A total of 300 febrile patients in National Hospital Abuja were tested for malaria and typhoid fever. Table 1 shows the distribution of malaria parasite in relation to age and sex of the febrile patients. The result indicates that 40.67% (122 patients) of the total population were positive for malaria parasite infection. 10-19 age groups have the highest prevalence rate of 57.69%. Also the male have the highest percentage of malaria infection of 44.27% relative to the female with 37.87% in the study sample.

Table 1 shows the distribution of typhoid fever in relation to age and sex of the patients. A total of 150 patients (50%) with significant Widal titre ($\geq 1/80$) results were obtained from the analysis. A high prevalence of 73.33% significant Widal titre result was found in the age group >60. The male had 50.38% of the significant Widal titre, and the female had 49.70%.

Table 3 shows malaria and typhoid fever coinfection in relation to age and sex of the patients. The result indicates that 27.33% co-infection with malaria and typhoid fever among patients. Female had the highest co-infection rate of 28.99% while the male had 25.19% rate. The age group of 10-19 had the highest co-infection rate of 46.15%.

Figure 1 represents a pie-chart showing percentage distribution of positive samples. Malaria infection has 40.67%, typhoid fever has 50% and coinfection has 27.33%.

Table 1 Distribution of malaria parasite in relation to age and sex of the febrile patients attending National Hospital Abuja

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Total No Examine</th>
<th>MALE No. Tested (%) Pos.</th>
<th>FEMALE No. Tested (%) Pos</th>
<th>Total/ (%) Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>73</td>
<td>42 (54.76)</td>
<td>31 (45.16)</td>
<td>37 (50.68)</td>
</tr>
<tr>
<td>10-19</td>
<td>26</td>
<td>10 (60)</td>
<td>16 (56.25)</td>
<td>15 (57.69)</td>
</tr>
<tr>
<td>20-29</td>
<td>63</td>
<td>22 (36.36)</td>
<td>41 (26.83)</td>
<td>19 (30.16)</td>
</tr>
<tr>
<td>30-39</td>
<td>56</td>
<td>17 (64.71)</td>
<td>39 (41.03)</td>
<td>27 (48.21)</td>
</tr>
<tr>
<td>Age group (Years)</td>
<td>Total No. Examine</td>
<td><strong>MALE</strong></td>
<td>(No. Tested) (%)Pos.</td>
<td><strong>FEMALE</strong></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>----------</td>
<td>-----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>0-9</td>
<td>73</td>
<td>42</td>
<td>19(45.24)</td>
<td>31</td>
</tr>
<tr>
<td>10-19</td>
<td>26</td>
<td>10</td>
<td>6(60.00)</td>
<td>16</td>
</tr>
<tr>
<td>20-29</td>
<td>63</td>
<td>22</td>
<td>14(63.64)</td>
<td>41</td>
</tr>
<tr>
<td>30-39</td>
<td>56</td>
<td>17</td>
<td>6(35.29)</td>
<td>39</td>
</tr>
<tr>
<td>40-49</td>
<td>39</td>
<td>20</td>
<td>8(40.00)</td>
<td>19</td>
</tr>
<tr>
<td>50-59</td>
<td>11</td>
<td>11</td>
<td>5(45.45)</td>
<td>17</td>
</tr>
<tr>
<td>&gt;60</td>
<td>9</td>
<td>9</td>
<td>8(88.89)</td>
<td>6</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>300</td>
<td>131</td>
<td>66(50.38)</td>
<td>169</td>
</tr>
</tbody>
</table>

Table 2 Distribution of typhoid fever in relation to age and sex of the patients attending National hospital Abuja

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Total No. Examine</th>
<th><strong>MALE</strong></th>
<th>(No. Tested) (%)Pos.</th>
<th><strong>FEMALE</strong></th>
<th>(No. Tested) (%)Pos.</th>
<th>Total/ (%)</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>73</td>
<td>42</td>
<td>12(28.57)</td>
<td>31</td>
<td>9(29.03)</td>
<td>21(28.77)</td>
<td></td>
</tr>
<tr>
<td>10-19</td>
<td>26</td>
<td>10</td>
<td>4(40.00)</td>
<td>16</td>
<td>8(50.000)</td>
<td>12(46.15)</td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>63</td>
<td>22</td>
<td>6(27.27)</td>
<td>41</td>
<td>6(14.63)</td>
<td>12(19.05)</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>56</td>
<td>17</td>
<td>4(23.53)</td>
<td>39</td>
<td>12(30.77)</td>
<td>16(28.57)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>39</td>
<td>20</td>
<td>0(0.00)</td>
<td>19</td>
<td>6(31.58)</td>
<td>6(15.38)</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>300</td>
<td>131</td>
<td>66(50.38)</td>
<td>169</td>
<td>84(49.70)</td>
<td>150(50)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Malaria and typhoid fever co-infection in relation to age and sex of the patients attending National Hospital Abuja
<table>
<thead>
<tr>
<th>Age Group</th>
<th>Malaria</th>
<th>Typhoid</th>
<th>Coinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-59</td>
<td>28</td>
<td>11</td>
<td>4 (36.36)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>15</td>
<td>9</td>
<td>3 (33.33)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>300</td>
<td>131</td>
<td>33 (25.19)</td>
</tr>
</tbody>
</table>

Figure 1 Pie-chart showing percentage distribution of positive samples
DISCUSSION
Malaria and typhoid fever can co-infect an individual causing a more severe illness. The frequent diagnosis of malaria, paratyphoid and typhoid disease in patients has made some physician to always assume the concurrent infection of the diseases. Hence, they always treat the two diseases concurrently without appropriate diagnosis. Such attitude towards drug administration can lead to increase in drug resistance to antibiotic and anti-malaria drugs and patient’s management.

Although, malaria and typhoid fever are said to be endemic in Nigeria, this study shows that co-infection in patients with malaria and typhoid fever has a rate of 27.33% and serological (widal) diagnosis of typhoid fever has a high infection rate of 50% as against the malaria infection which has a total infection rate of 40.67%.

According to this study, salmonella serotypes were evaluated based on their high antibody titre. *Salmonella* serotypes including *S. typhi*, *S. paratyphi A*, *S. Paratyphi B* and *S. paratyphi C* are bacterial causative agents of typhoid fever and its similar paratyphoidal fever [20]. Antibody titre of, 1/80 and above was taken as significant titre. The titre is chosen based on similar significant titre that have been established in areas and regions around the study area where the patients are coming from [13, 16].

The infection rate using Widal test was higher between the ages of ≥60 (73.33%). This high infectivity amongst adult shows that it is not only high in children but could be high in adults contrary to studies that typhoid fever is higher in ages of 5 years and below [1]. Also the result for the Widal test in relation to gender showed that the male had a higher number of positive samples (50.38%) than the female (49.70%). This is in agreement to previous studies that Typhoid fever is more frequent in males, and this is a notion based on observations made in patients hospitalized with typhoid fever [18, 6]. Chi-square analysis revealed that typhoid fever is significant amongst febrile patients (table 2).

Test for malaria was higher between the ages of 10-19 (57.69%). This is contrary to the reports from Awka, Anambra State, which had the highest of 58.3% between the ages of 0-5 years [11]. The present study showed that Plasmodium infection is more common in male (44.27%) than in female (37.87%). Studies have shown that females have better immunity to parasitic disease and this was attributed to genetic and hormonal factors [17]. Chi-square analysis revealed that malaria fever is significant amongst febrile patients (table 2).

This study has shown that typhoid fever and malaria co-infection was 82 (27.33%) using widal agglutination test (table 3). This was in agreement with the work of [12, 3], where they had 0.5 (10.1%) and 17(47.9%). Chi-square analysis reveals that typhoid fever is significant amongst febrile patients attending National Hospital, Abuja (table 3).

CONCLUSION
Typhoid fever and/or malaria is a major public health problem in Nigeria. From the investigation carried out, the prevalence of typhoid fever is 50%, malaria is 40.67% and their co-infection is 27.33%. The Chi-square value for the investigation showed that typhoid, malaria coinfection is significant amongst patients.

RECOMMENDATION
The following recommendations can be emphasized to serve as preventive and control measures for malaria and typhoid fever infection in the study area:
1. Due to unreliability of widal agglutination test, positivity has been associated with non-typhoid fever resulting from cross-reacting antibodies, malaria, cirrhosis and hepatitis, efforts should be made to find more accurate and specific clinical and cultural methods of identifying and isolating *Salmonella* species in specimens.
2. Proper laboratory tests should be carried out on patients before the administration of drugs so as to avoid drug resistance.
3. Further studies should be done on the other potential risk factors of malaria and typhoid fever co-infection in different seasons and different study areas.
4. The continued development of better diagnostic tools for both malaria and typhoid fever is still crucial.

REFERENCES


