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## Articles



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# Studies on Co-Infection of Plasmodium falciparum and Salmonella Spp. in Ota, Ogun State, Nigeria

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***Abstract***: Salmonella and Plasmodium infections are major health challenges especially in regions where malaria is highly endemic. Studies were carried out to determine the incidence of co-infection of Salmonella spp and Plasmodium falciparum among subjects that present with fever at the Covenant University Health Centre and Ota General Hospital between September, 2011 and May, 2012. Salmonella infection was detected by comparing two diagnostic methods: serology and culture on the blood samples collected. Widal test was carried out by detecting the 'O' and 'H' antigens in the blood and the blood samples were cultured using Thioglycolate broth and Salmonella Shigella agar. Plasmodium infection was confirmed through microscopic examination of Giemsa stained thick and thin films of the same blood samples. Out of the 84 samples collected, 45.2% was positive for Salmonella and Plasmodium co-infection by Widal test with positive titre ≥ 1/80. Only 3.6% was confirmed for co-infection of Salmonella and Plasmodium Species when Salmonella infection was detected by culture. Among the 84 subjects 73.8% was positive for malaria alone and 67.9% for Salmonella infection alone. Laboratory confirmation of co-infection of malaria and Salmonella is essential to prevent wrong treatment and misdiagnosis.

***Keywords***: Co-infection, Incidence, Salmonella, Plasmodium

## Introduction

Malaria and typhoid fevers remain as threats to so many people for several reasons: the increasing poverty, deterioration in public health services, compounded HIV/AIDS and increasing resistance of malaria parasites to antimalarial drugs (Olasehinde, 2010; WHO, 2010), lack of potable water (Olasehinde *et al*., 2013) and widespread misuse of Widal agglutination test for diagnosing typhoid fever (Pang, 1989), increased requests for Widal test as a means of making money by private laboratories are other factors (Usman, 2002).

Malaria, a tropical disease of man characterised by fever, malaise and weakness. It causes incidence estimates of 2 to 3 million deaths and 300 to 500 million clinical cases in

the world (Nikura *et al*., 2008). The vast majority of cases occur in children under the age of five years and pregnant women (Olasehinde *et al*., 2010). The disease is caused by the protozoan parasite belonging to the genus *Plasmodium*. *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* are the five species known to cause disease in man. Infection with *P. falciparum*, the most virulent species (WHO, 2010) is associated with developing fever, high number of parasites in the blood and pathogenesis including severe anaemia, body weight loss and cerebral malaria in humans (Nikura *et al*., 2008, Olasehinde *et al*., 2014). Malaria is transmitted through the bite of an infected female *Anopheles* mosquito.

Typhoid fever is an acute systemic infection caused by the bacterium *Salmonella enterica* sub-sp*enterica* serotype *typhi* (or simply *Salmonella typhi*). It is transmitted by fecal-oral route via contaminated food and water. An estimated of 17 million cases of typhoid are reported worldwide each year, resulting in 0.6 million deaths (Sulaimon, 2006). This is exacerbated by the emergence and spread of multidrug-resistant strains of *Salmonella typhi*, and further complication by malaria co-infection (Bhan *et al.*, 2005).

Malaria and typhoid fevers are among the most endemic diseases in the tropics (Opara *et al*., 2011, Uneke, 2012). Both diseases have been associated with increasing poverty, deterioration in sanitation, poor public health services, compounded with increasing drug resistance of the two aetiological agents (Alnwich, 2001).

Although the two infections are caused by very different agents and transmitted via different mechanisms, both diseases share rather similar symptoms (Uneke *et al.*, 2008, Agwu *et al., 2009*). This presents a challenge of diagnostic error. Definitive laboratory-based diagnosis is thus required to differentiate the two infections as well as detect co-infections.

## Materials and Methods

### Study Area and Subject

This study was a cross-sectional study. Samples were collected from Covenant University Health Centre and General Hospital Ota, Ogun State. Participants were included among patients visiting the Out-Patient Department of the two hospitals. In all, eighty four (84) participants were sampled for the study.

### Collection and Analysis of Samples

Three milliliters (3ml) of blood sample was collected from each patient into heparinized bottles by trained and licensed medical laboratory technologists from the two hospitals. Ethical permit was given by Nigerian Institute of Medical Research (NIMR) and Ogun State Hospitals Management Board before this study was conducted.

The Widal agglutination test was

performed on all blood samples by the rapid slide test using Micropath Antigens/Febrile Antigen Kits (Omega Diagnostic LTD, UK) for the somatic (O) and flagella (H) antigens. The rapid slide test was used as a primary screening procedure.

**Blood culture**

Two milliliters of each blood sample were aseptically introduced into 18 ml of Thioglycolate broth and incubated at 37°C for an initial period of 48 h and sub-cultured on *Salmonella Shigella* agar (Lab M). *S. typhi* organisms were identified on the basis of standard cultural, microscopic and biochemical characterization. Inoculated blood culture media was discarded as negative if there was no growth after 7-10 days.

**Identification of Isolates**

The pure isolates were stained according to Gram's techniques as described by Cheesbrough (2005). Biochemical tests such as Indole, urease, citrate utilization, sugar fermentation and oxidase tests were carried out to characterize and identify the isolated organisms.

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**Results**

The result of this study is based on parasitological examination for malaria parasites and bacteriological and serological tests for the diagnosis of typhoid fever in 84 patients attending Covenant University Health Centre and General Hospital Ota. The patients comprised of 36 males and 48 females. Table 1 shows the percentage incidence of co-infection of *Salmonella spp* and *Plasmodium falciparum* with age group >26 having the highest incidence. Table 2 shows samples positive for *Plasmodium falciparum* alone. Table 3 shows the incidence of *Salmonella* infection, fifty-seven out of the 84 samples were positive for typhoid by the Widal test (serology) considering a positive Widal test for any sample showing antibody titre of greater or equal to 1 in 80 while Table 4 shows that five samples were positive for typhoid fever by blood culture. Table 5 shows the distribution of antibody titres against *Salmonella spp.* Table 6 shows the biochemical characteristics of the isolates

Table 1. Co-Infection of *Plasmodium falciparum* and *Salmonella spp.*

| S/N | Age Range | No. of Samples Collected (%) | | | No. of Positive Samples | | Total (%) |
|---|---|---|---|---|---|---|---|
| | | M | F | Total | M | F | |
| 1 | 0-5 | 4 | 2 | 6 | 1 | 1 | 2 (5) |
| 2 | 6-10 | 1 | 5 | 6 | 1 | 0 | 1 (3) |
| 3 | 1I-15 | 2 | 4 | 6 | 1 | 2 | 3 (8) |
| 4 | 16-20 | 10 | 12 | 22 | 6 | 7 | 13 (34) |
| 5 | 21-25 | 4 | 5 | 9 | 0 | 4 | 4 (11) |
| 6 | >26 | 15 | 20 | 35 | 7 | 8 | 15 (15) |
| Total (%) | | 36 (43) | 48 (57) | 84 (100) | 16 | 22 | 38 (45) |

Table 2. Incidence of *Plasmodium falciparum* Infection in Ota

| S/N | Age range (Yrs) | No. of Samples Collected | | | No. of Positive Samples | | |
|---|---|---|---|---|---|---|---|
| | | M | F | Total | M | F | Total (%) |
| 1 | 0-5 | 4 | 2 | 6 | 3 | 2 | 5 (8) |
| 2 | 6-10 | 1 | 5 | 6 | 1 | 2 | 3 (5) |
| 3 | 11-15 | 2 | 4 | 6 | 1 | 3 | 4 (6) |
| 4 | 16-20 | 10 | 12 | 22 | 9 | 10 | 19 (31) |
| 5 | 21-25 | 4 | 5 | 9 | 3 | 5 | 8 (13) |
| 6 | >26 | 15 | 20 | 35 | 10 | 13 | 23 (37) |
| Total (%) | | 36 | 48 | 84 (100) | 27 | 35 | 62 (73.8) |

Table 3. Incidence of *Salmonella* Infection by the Widal Test (serology)

| S/N | Age Range (Years) | No. of Samples Collected | | | No of Positive Samples (Serology) | | | Percentag |
|---|---|---|---|---|---|---|---|---|
| | | M | F | TOTAL | M | F | TOTAL | |
| 1 | 0-5 | 4 | 2 | 6 | 1 | 1 | 2 | 4% |
| 2 | 6-10 | 1 | 5 | 6 | 1 | 1 | 2 | 4% |
| 3 | 11-15 | 2 | 4 | 6 | 2 | 3 | 5 | 9% |
| 4 | 16-20 | 10 | 12 | 22 | 8 | 8 | 16 | 28% |
| 5 | 21-25 | 4 | 5 | 9 | 1 | 4 | 5 | 8% |
| 6 | >26 | 15 | 20 | 35 | 12 | 15 | 27 | 47% |
| Total | | 36 | 48 | 84 | | | | 100% |

Table 4. Incidence of *Salmonella* Infection Confirmed by Culture Method

| S/N | Age Range (Years) | No of Positive Samples (Blood Culture) | | |
|---|---|---|---|---|
| | | M | F | Total |
| 1 | 0-5 | - | - | - |
| 2 | 6-10 | - | - | - |
| 3 | 11-15 | - | 1 | 1 |
| 4 | 16-20 | - | - | - |
| 5 | 21-25 | - | - | - |
| 6 | >26 | 2 | 2 | 4 |
| Total | | 2 | 3 | 5 |

Table 5. Distribution of *Salmonella* Antibodies

| Age Range | M | | | | F | | | |
|---|---|---|---|---|---|---|---|---|
| | | 80 | 160 | T | | 80 | 160 | Total |
| 0-5 | O | 1 | - | 1 | O | - | - | - |
| | H | - | - | - | H | 1 | 1 | 1 |
| 6-10 | O | - | 1 | 1 | O | 1 | 1 | 1 |
| | H | 1 | - | 1 | H | 1 | 1 | 1 |
| 11-15 | O | - | 1 | 1 | O | 2 | 2 | 2 |
| | H | - | 1 | 1 | H | 1 | 1 | 2 |
| 16-20 | O | 1 | 2 | 3 | O | 4 | 4 | 6 |
| | H | 3 | 6 | 9 | H | 5 | 5 | 10 |
| 21-25 | O | 1 | - | 1 | O | 3 | 3 | 4 |
| | H | - | - | - | H | 1 | 1 | 2 |
| >26 | O | 4 | 8 | 12 | O | 6 | 6 | 11 |
| | H | 7 | 7 | 14 | H | 13 | 13 | 17 |

O = Somatic antigen of *S. typhi*
H = Flagella antigen of *S. typhi*

Table 6. Biochemical Characteristics of *Salmonella* Isolates

| Sample Code | Citrate | Urease | Indole | Oxidase | Sugar Fermentation | | | Suspected SPP. |
|---|---|---|---|---|---|---|---|---|
| | | | | | GLU | LAC | SUC | |
| 1 | + | _ | _ | _ | +G | _ | _ | *Salmonella spp* |
| 2 | _ | _ | _ | _ | +G | _ | _ | *Salmonella spp* |
| 3 | + | _ | _ | _ | +G | _ | _ | *Salmonella spp* |
| 4 | _ | _ | _ | _ | +G | _ | _ | *Salmonella spp* |
| 5 | _ | _ | _ | _ | +G | _ | _ | *Salmonella spp* |

## Discussion

The incidence of co-infection of *Plasmodium falciparum* and *Salmonella* infections in Ota was detected in this study. Findings in this study show that 67% of the samples were positive for antibody titres against *Salmonella* serotypes. Only 6% incidence rate was recorded for *Salmonella* infection when the blood samples were cultured. The isolation of *Salmonella* species for detection of their O and H antigens from blood samples and confirmation of malaria parasites in blood samples are confirmatory tests for typhoid/paratyphoid and malaria infections (WHO, 2003).

The incidence rate of co-infection of typhoid/paratyphoid fever and malaria by serological diagnosis of *Salmonella* infection was 45.2% (Table 3) while the incidence of co-infection by culture of the blood samples was 6% (Table 4). The results of incidence of *Salmonella* infections in this study confirmed the claims that higher incidence rates are observed when serological methods are used than when cultural methods are employed. Eze *et al.,* (2011) reported a similar result of 48% incidence by serology of samples that were not positive by culture. The difference in the results of detection of *Salmonella* infections by culture and serology may depend on individual host immune responses, which become stimulated in febrile conditions associated with malaria fever (Eze *et al*., 2011). This memory response could cause positive Widal reactions in previously sensit 6 patients. Also similar studies by Mbuh *et al. (*2003) reported the high rate of typhoid and malaria co-infection associated with the Widal test and the blood cultural results showed that this rate of co-infection could be reduced to only 0.5%.

The incidence rate of *Plasmodium falciparum* infection alone in this study was 73.8% while that of *Salmonella* infection was 67.9% alone. The incidence rate in this study is lower than that observed in earlier studies by Olasehinde *et al*. (2010) where a prevalence rate of 80.5% was recorded among infants and children 0-12years old in Ota, Ogun state. The reduction in the incidence of malaria infection observed in this study may be associated with increase in awareness on the effective use of drugs and treatment of malaria among the population, improved standard of living and the widespread use of Long Lasting Insecticide Treated mosquito nets (LLIN) and insecticides. There was also an observed reduction in the incidence of *Salmonella* infection when compared with earlier studies on the incidence of salmonella infections in Borno and Plateau states of Nigeria where Mohammed *et al.* (1992) found a reciprocal O and H antibody titres in 92.7% and 90.7% respectively. The reduction may also

be as a result of improved proper hygiene and community health education public health measures that could help to prevent and control typhoid fever (Sur *et al*., 2006).

In co-infections, the diagnosis of typhoid should be made from a culture specimen as false positives and overestimation occur with the use of the Widal test. Ammah *et al.* (1999) reported that out of 200 patients with fever, 17% had concurrent malaria and typhoid fever based on bacteriological proven diagnosis as compared to 47.9% based on the Widal test. In this study 45.2% of the subjects had concurrent antibody titres against *Salmonella* serotypes and malaria and 6% has malaria and typhoid fever based on cultural method. This is to be expected as the Widal test being a serological test, only proves exposure to a certain antigen. It does not tell if an infection is recent or not. Samal *et al.* (1991) described 52 patients with malaria positive in the peripheral blood smear (cases consisted of *vivax, falciparum* or mixed *vivax and falciparum*), out of whom eight cases had a positive Widal test but blood cultures were negative for *S.typhi* in all. The Findings in this study strongly suggest the inappropriateness of the use of widal test only as a diagnostic tool for *Salmonella* infections, since other infections can influence antibody titre against *Salmonella* serotypes. The antibody titre elevation could be as a result of cross reactivity of the antibody with the *Salmonella* antigens and that malaria infections cannot be associated with typhoid infections, though there could be co-infections.This will also improve patient management by cutting down cost of treatment and eliminate other risks associated with misuse of antibiotics. One of the factors that affect the reliability of diagnosis by culture method is uncontrolled use of antibiotics before case reports at the hospitals. Misdiagnosis and development of resistance among pathogenic organisms have been associated with uncontrolled use of drugs and incomplete dosage and increased consumption of drugs (Marks *et al*., 2005).

## Conclusion

The incidence of co-infection of *Salmonella spp* and *Plasmodium falciparum* in Ota, Ogun state, Nigeria has been established in this study, culture and microscopy have been found to be more useful in the determination of malaria and *Salmonella* infections.

While novel ‘point of care’ quick diagnostic methods for malaria and Salmonella infections are being developed, we recommend that appropriate and complete laboratory diagnostic procedures be followed. This will reduce misdiagnosis and ensure adequate treatment of both infections, especially in areas where malaria is endemic.

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