Efficacy of Some Anti-Malaria Drugs and Antibiotics on Plasmodium and Salmonella Species Isolated From Patients Attending A University Health Center in Nigeria

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Abstract

Background*: Malaria is the highest killer of people in Africa, and Nigeria in particular. It kills more people than the deadly human immunodeficiency virus, (HIV). In fever investigation, two important diagnoses to be ruled out are typhoid and malaria. An association between malaria and typhoid fever was established and named "typho-malaria fever".*

Method: *Blood and stool specimens were collected from a total of 200 male and female patients aged 5-60 years attending University of Jos Health centre. Apparently healthy individuals (controls) and patients with symptoms of fever were examined for malaria parasitaemia and salmonella species by microscopy and stool/blood culture. P-alaxin, Artemether/Lumefantrine, Artesunate/Amodiaquine, and ciprofloxacin, Cefuroxime &Azithromycin were the anti-malaria and anti-biotic drugs used respectively. The microscopic film for positive malaria parasite cases and the sensitivity of the Salmonella isolates were recorded.*

Results: *A total of 90 out of 200 (45.0%) specimen were positive for the 1st malaria test (pre-administered results) and 39 (19.5%) were positive for 2nd malaria test (post-administered results). While 12 out of 200 (6.0%) subjects' yielded Salmonella species. The data obtained which were subjected to statistical analysis using ANOVA at (p<0.05) and chi square test.*

Conclusion: *Densely populated malaria parasite mean showed anti-malaria drug resistance in vivo, while cultural isolated Salmonella organism showed precise drug sensitivity invitro. In other words, heavy & multiple re-infection of malaria parasite exhibited some degree of resistance to the anti-malarial drugs while salmonella in vitro culture results represented sensitivity to the anti-typhoid drugs used.*

Keywords: *Malaria, Typhoid fever, anti-malaria, antibiotics, efficacy.*

I. Introduction

Malaria and typhoid fever are among the most endemic diseases in Africa. In the investigation and diagnosis of fever in Nigeria, two important diagnoses to be ruled out are malaria and typhoid. Both diseases have been associated with poverty and under development with significant death rates [1]. An association between malaria and typhoid fever was described in the medical literature in the United States Army [2].

Malaria is one of the most serious health problems facing the world today. The World Health Organization estimates that over 300 million new cases and approximately 2-3 million deaths of malaria occur each year. Malaria is endemic in tropical Africa, with an estimated 90% of the total malaria incidence and deaths occurring in the continent, particularly among pregnant women and children.

Malaria is a mosquito –borne infectious disease caused by a eukaryotic protist of the genus plasmodium. There are currently six species of genus *Plasmodium* known to infect humans; *Plasmodium falciparum*, *P.vivax*, *P. ovale*, *P. curtisi*, *P. ovale walker*, *P. malariae* and *P. knowlesi* ([3][4][5]). *Plasmodium falciparum* is the species responsible for most of the mortality and morbidity associated with the disease. Malaria and typhoid fever often present with mimicking symptoms especially in their early stages. These include; fever, headache, vomiting, stomach-ache and body pain. The situation often presents a diagnostic confusion [6]. It is sometimes difficult to differentiate clinically, the presentation of typhoid fever from that of malaria without laboratory support and diagnosis. However, differentiation can be achieved using cultural laboratory diagnostic tests [7]. According to [8] in a study publication on epidemiological studies of malaria and typhoid infections in Abeokuta, Ogun State Nigeria, the prevalence of typhoid infection was 17.4% but did not differ between sexes although significantly higher among older children (P=0.0001). Their observation from the longitudinal study carried out between October 2000 and September 2001 in Abeokuta, Ogun State Nigeria showed that out of the 3997 feverish cases recorded, 82.4%and 32.8% were respectively due to malaria and typhoid.

Worldwide drug resistance constitutes an impediment to the control of malaria [9]. This has led to the need for constant surveillance and monitoring for changes in the sensitivity of malaria parasites to different malaria drugs [10]. The effect of anti-malaria drug is generally characterized by the inhibition of parasite growth and consequently the parasite is multiplied. As a result of widespread resistance, malaria therapy now consist combination of two or more drugs that attack different biochemical processes in plasmodium [11]. Recently, the federal ministry of Health Nigeria has implemented the new treatment policy on malaria which includes the use of Artemisinin-based combination therapy (ACT) as the new first line drug for the treatment of uncomplicated malaria [12]. New discovery could help turn antibiotic into anti malaria drugs. This will go a long way in alleviating the problems faced with drug resistance which is in line with Melbourne researchers making progress towards new anti-malaria drugs. This was after revealing how an antibiotic called emetine blocks the molecular machinery that produces the protein required for malaria parasite survival [13]. This study was carried out to determine the efficacy of some common malaria drug and antibiotics on patients in terms of drug sensitivity. More studies have proved that high prevalence of *malaria* is an established fact. Furthermore, within the last decade an unusually high number of illnesses have been diagnosed as malaria coexisting with typhoid fever [4].This established fact of co-existence of malaria and typhoid has led to this present study in search of one drug that will eliminate, if not eradicate these co-existing organism.

Typhoid and paratyphoid fever are caused by *Salmonella* species which are water borne disease that causes infection of the intestinal tract and blood streams, [14].
An estimated 17 million cases of typhoid were reported worldwide each year, resulting in 0.6 million deaths [15]. The main route of transmission is by person-to-person spread, either in hospitals or in community. The incubation period of enteric fever bacilli is usually 10 – 20 days, with diarrhea, starting 3 – 4 days after onset of fever and lasting an average of 6 days. ([16],[17]). Asymptomatic persons may also harbor *Salmonella* unknowingly. Both remains potentially infectious for weeks or months some become infectious and some becomes life-long excreters. Although typhoid and *malaria* are caused by two very different organisms: – gram negative bacilli (*Salmonellae*) and a protozoan (Plasmodium) .They are transmitted via different mechanisms. This two important diseases share rather similar symptomatology. *Malaria* and typhoid fever often present with mimicking symptoms especially in the early stages of typhoid fever. The similar symptoms include; fever, headache, vomiting, stomach ache and body pain. The situation often presents a diagnostic confusion. [4]. However, it is sometimes very difficult to differentiate clinically, the presentation of typhoid fever from that of *malaria* without laboratory support and diagnosis.

II.   Materials And Method

Study Area/Location
The study was conducted in the University of Jos Clinic (UHS) located in Jos North Local Government Area of Plateau State, Nigeria. The Clinic serves the general medical needs of the entire University Staff, Students, and Staff dependants, patients living in and around the University environment.

Study population:
A Total of 200 patients (male & female) comprising of in- patients presenting at the clinic with signs and symptoms suggestive of typhoid fever or malaria symptoms and 50 apparently healthy individuals visiting the Clinic for other health/ related purposes were recruited for the study. They were aged 5-60 years.

Ethical consideration:
The protocol for the study was reviewed and approved by Ethical Review Committee of Jos University Teaching Hospital (JUTH) Jos Nigeria. In addition, informed consent was sought and obtained from the Patients or Parents of children. A structured questionnaire was used to obtain the biodata of the participants.

Collection of specimens:
Samples used in this study were blood and stool samples. Five milliliter (5ml) of blood samples were aseptically collected by vein- puncture. Stool specimens were collected into clean wide mouth glass bottles.

Processing of specimens; Preparation of blood films:
A drop measuring six microlitre (6μl) of blood was placed on a clean grease free glass slide, to make a thick blood film by spreading it onto the glass slide. This was allowed to air dry and stained using fields stain A & B.

The thin blood film was made by placing a drop measuring 2μl drop of blood onto a grease free glass slide and a blood spreader placed to touch the blood at an angle of 45^0C. With a quick and fast movement, the blood was spread onto the slide to have a head, body and tail and allowed to air dry and stained using Leishman stain [18].

Stool:

Pea size of the stool sample (About 1gm) was inoculated into 10mls of Selenite F (liquid media) and incubated at 37^0C for 24hrs. Stool samples were cultured following standard procedures for Salmonella species' isolation as stated in [19].This was further sub cultured onto Deoxycholate citrate agar (DCA) and Salmonella Shegella (SS) agar. They were also incubated for 24hours at37^0C. The standard method of inoculating plate and the pour plate method of setting up sensitivity as stated in the Medical Laboratory Manual For Tropical Countries were used. Salmonella species were identified on the basis of cultural, microscopic and biochemical characterization ([19][20]).

Separation of serum:

The two milliliter (2mls) of the blood specimens was allowed to clot and spun for 5 minutes at 2500 revolution per minute (rpm). After retracting, the Serum was separated and stored in the fridge until required.

III.   Laboratory Analysis

Parasitological examination of slides for Malaria parasite:

The thick and thin films were examined under the X 100 (oil emersion) power objective for the presence of any peripheral parasitaemia. Microscopically confirmed Malaria parasite subjects (pre-drug test) were administered anti-malaria drugs. Patients administered antimalarial drugs (Artemether/Lumefantrine; Artesunate Amodiaquine; P-Alaxin) were rescreened for malaria parasitaemia after four days to re-determine the efficacy of the drugs.

Serological Test for Typhoid fever:

The sera were screened for antibodies to *Salmonella* using the commercial antigen suspension reagents (Biosystem, febrile Serodiagnostics). A drop of the serum was placed on a tile mixed with a drop of the antigen, rocked in a mixer for 3-4 minutes and observed for agglutination. Significant widal tests were recorded when the somatic (0) and flagella (H) gave agglutination reaction tiles of $\geq {}^1/_{80}$.

Bacteriological Test for Typhoid:
Stool Culture:

The stool sample inoculated into 10mls of Selenite f and incubated aerobically at 37^0C for 24hrs was plated out onto these solid media. This was aseptically sub cultured into   Deoxycholate citrate agar (DCA) and *Salmonella –Shigella* agar (SSA) using a sterile wire loop, a loop full was picked, sub cultured onto the media and incubated aerobically at 37^0C for 24hrs after which they were observed for growth. Suspected *Salmonella* colonies were identified as *Salmonella* enteric serovar typhi using standard microbiological techniques [21].

Blood Culture:

Two milliliter of the blood samples were inoculated into 18mls of prepared brain heart infusion broth and mixed by rotating in between the palms for 1 minute. These were then incubated aerobically at 37^0c for 24hrs. This was later sub-cultured onto *Salmonella Shigella* agar using a sterile wire loop and incubated aerobically at 37^0c for 24hrs. The brain heart infusion broth media were further incubated for another eight days after which any slow growing organism will be isolated by further sub-culturing as above. The identified *Salmonella* bacilli were set up for drug sensitivity testing (Ciprofloxacin; Cefuroxime and Azithromycin drugs tested in the sensitivity single disc prepared).

Sensitivity Testing:

Pour plate method for sensitivity testing: The isolated salmonella Spp were inoculated into 10mls of peptone water and incubated at 37^0c for 2hrs before pouring onto the dry Mueller Hinton sensitivity agar media plate. Then the excess was drained off from the top of the agar media plate. The already prepared antibiotic single disc were placed onto the plate and incubated aerobically at 37^0c for 24hrs. The zone of clearance were measured and recorded in millimeters. The three single disc used were; Ciprofloxacin, Azithromycine and Cefuroxime.

IV.   Results

Table 1 represents the first microscopic laboratory examination of malaria parasites and widal screening test for Febrile Patients and control subjects. Ninety (45.0%) of the febrile and fifteen (30.0%) of the control subjects out of the two hundred study subject and fifty control subjects respectively were positive for malaria parasites and widal for typhoid. Those subjects with significant titer of $\geq {}^1/80$ and positive for malaria otherwise called typhomalaria fever cases were 110 (55.0%) and 20 (40.0%) of the study control subjects out of 200 and 50 cases respectively.

The "real gold" standard test of cultural isolated salmonella organism and microscopic malaria parasite positive cases were 102 (51.0%) and 23(46.0%) for the control subjects

Table 1: First Microscopic Examination of Malaria Parasite (MP), Widal Screening, Culture Isolated Salmonella species and Control Subjects.

Infection	Subjects (%)	
	Febrile Patient N= 200	Control Subjects N= 50
Positive for malaria parasite (MP)	90(45.0)	15(30.0)
Positive for widal + MP	110 (55.0)	20(40.0)
Positive for MP + Isolated Salmonella Spp.	102 (51.0)	23(46.0)
Negative for MP &Cultures for Salmonella Spp.	90 (45.0)	27(54.0)
Total	200(100)	50(100)

Table 2 shows the results of second microscopic examination of malaria parasites and sensitivity pattern of isolated salmonella species. Four days after the subjects have taken antimalarial Drugs, their blood samples were retested (post drug test) for MP, microscopically confirmed Positive cases were 39 out of 90 positive cases of pre drug test. Eight out of the fifteen (pre drug test) subjects of the control were also positive for post drug mp test. Twelve *Salmonella* isolated subjects showed sensitivity pattern by inhibition clearance zones of 2-4 milliliter for ciprofloxacin being the most sensitive drug, followed by 10 subjects for ceftrioxone and 8 for Azithromycin, while 7 subjects showed very minimal sensitivity or resistance to the drugs used in this assay.

Table 2: Second microscopic examination results for *Malaria* parasite and sensitivity tests for isolated *Salmonella* spp.

Infection	Subjects (%) Typhomalarial fever	
	Febrile	Control
Positive for malaria parasite only	39(38.2)	8(34.78)
Salmonella Isolate sensitive to *Cip. (2 – 4mm)	12(11.76)	4(17.39)
Salmonella Isolate sensitive to *Cef. (2 – 3mm)	10(9.8)	1(4.34)
Salmonella Isolate sensitive to *Azith. (2mm)	8(7.84)	1(4.34)
Salmonella Isolate of sensitivity less than 2mm	9(8.82)	2(8.69)
Negative for MP	26(25.49)	7(30.43)
Total	102(100)	23(100)

* – Ciprofloxacin=cip, Cefuroxime=cef, Azithromycine-Azith.

V. Discussion

Epidemiological observation suggests that strategies that control *Malaria* also decrease the rates of bacterial infections & multiplications [22].Although malaria and typhoid are said to be endemic in Nigeria, this study shows that *Malaria* is far more likely to cause fever than typhoid fever. [23] observed the malaria high prevalence and reliability of cultural isolation of *salmonella especially* in invitro drug sensitivity. A reliable malaria parasite result can be established from a confirmed microscopic positive test .This is in agreement that microscopy is the" gold standard" for malaria parasite diagnosis.([24][25]).This has helped to rule out the confusion faced by clinicians in handling Typhomalarial cases. Cultural diagnosis and isolation of *typhoid* fever shows the unreliable nature of widal test, which is basically used in the diagnosis of *typhoid* fever in the Tropics and Nigeria in particular. A reliable diagnosis of typhoid fever is based on cultural isolation of *Salmonella* organism ([26],[20]). This study is in line with the publications of Pearson *et al.,* [27] and Prasanna [25] and 2000 confirming Blood culture as the gold standard for typhoid diagnosis. Also in line with that of Prasanna [25] that provided information on *malaria* and *typhoid* co-infection otherwise named *'typhomalarial* fever' being a major public health problem. Patients presenting with fever clinically suspected to be either malaria or typhoid should be subjected to laboratory diagnosis for confirmatory tests. Early results confirming *malaria* positive and *salmonella* Sensitivity to antibiotics should be administered with the sensitive drugs, in order to prevent drug resistance and other side effects, faced by self medication that can lead to many deaths / casualties.

In conclusion, the result in this study indicates that Artemisinin drugs are the best for the treatment of uncomplicated malaria infection, especially in co- infections with malaria. This will further led to the eradication of the bacteria from the body. All these are in agreement with the World Health Organization (WHO) [24], recommending that ACT is the first line treatment of malaria infection. Today, WHO recommends Artemisinin –based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by *P. falciparum.* ACTs has been integral to the remarkable recent success in global malaria control, and there is blood consensus that protecting the efficacy of these ACT medicine combinations is an urgent priority [24]. The most effective drug established from this study being Artemether/Lumefantrine, this concurring with the publications of Donelly *et al.,* [5]. Furthermore, the prompt treatment of typhoid disease with antibiotics