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Prevalence of Plasmodium and Salmonella Species from Patients Attending University Clinic and Hospital in Nigeria

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Abstract: ***Background:*** *Since 2013 till date, Malaria and Typhoid fever has been the major cause of fever for consultation in health centers. Epidemiological, Cultural and Drug Sensitivity Prospective study spanning 4 years was conducted in Unijos Health Services, this study purposed to describe and document the findings of the epidemiology and drug sensitivity pattern of Plasmodium species and Salmonellosis as evidence base data for the eradication of Malaria and Typhoid fever in Nigeria and the world at large.* ***Method:*** *Two hundred and fifty patient's blood and stools samples were diagnosed; age, gender, microscopy, culture and sensitivity were the variables, the drug effects of P-Alaxin, Artemether Lumefantrine and Artesunate Amodiaquine was determined. Similarly, the in-vitro effects of ciprofloxacin, cefuroxime and azithromycin and their synergic activities also determined.* ***Result:*** *The prevalence of Plasmodium and Salmonella species studied, unveiled the malaria positive cases of 193 (77.2%) while cases of Typhoid recorded 59 (23.6%). The age group of 16-26 years reviled highest incidence in both cases.* ***Conclusion:*** *Fever caused by malaria and typhoid was highest among female young adult, these representing strong evidence in addition with microscopic and cultural results obtained. Artemether Lumefantrine and ciprofloxacin were discovered to be the most effective, even exhibiting drug curative synergistic action. The ANOV reviled significant statistical difference (P < 0.05) among the drugs used.*

Keywords: *Drug effects, Prevalence, Plasmodium, Salmonella.*

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I. Introduction

Malaria ("bad air") is caused by obligate intracellular parasitic protozoan belonging to the genus *Plasmodium*. It is the most important infectious disease in the world [1]. Malaria is one of the most significant causes of morbidity and mortality worldwide ([2][23]). Globally, 3.2 billion people in 97 countries and territories are at risk of being infected with malaria and developing disease. In 2014, 198 million cases of malaria occurred, causing 584,000 deaths, especially in sub-Saharan African countries [3]. There are currently six species of the genus *Plasmodium* known to cause disease in man; *Plasmodium falciparum, P. knowlesi, P. malariae, P. ovale curtisi, P.ovale walkeri and P. vivax* [24]. *Malaria* is transmitted through the bite of an infected female Anopheles mosquito to man .Transmission infrequently occurs through blood transfusion, bone marrow transfusion and transplacentally. The infectious stages, the sporozoites may be injected in a single mosquito bite ([4][24]). This important parasite lives in human and feeds solely on human blood, reproducing both sexually and asexually in the human body. Worldwide drug resistance constitutes an impediment to the control of malaria [5]. This has led to the need for constant surveillance and monitoring for changes in the sensitivity of malaria to different anti malaria drugs. The current World Health Organization (WHO) guideline for the treatment of malaria recommended the use of Artemisinin-based combination therapy (ACT) for the treatment of malaria owing to Plasmodium resistance to monotherapy drugs [10]. Artemisinin containing combination therapies will increase in their usage and perhaps in their public health importance with time as resistance becomes more widespread to a wide range of single anti-malarial drugs [7].

Like malaria, there is a popular believe that typhoid fever is also endemic and quite prevalent in Nigeria ([8][9]). Typhoid and paratyphoid fevers are commonly grouped together under the collective term 'enteric fever'. Typhoid is caused by *Salmonella typhi* (strictly termed *S.enterica* sub-species *enterica serotypetyphi*) and paratyphoid is caused by either *Salmonella paratyphi A, B,* or *C. Salmonella paratyphi B* is also known as *S. schottmuelleri*, and *Salmonella paratyphi C know* as *S. hirschfeldii* [11].Both diseases are an indication of neglect of control of the environment. While both diseases and most especially typhoid are going extinct in the wilder world, there cases in Africa remain alarming as they have been recorded to constitute a major case of hospital admissions in Africa ([6][13]). It afflicts local inhabitants as well as travelers to endemic areas. Enteric fever is endemic in Nepal. *S. enteric* serotype Typhi and *S. enteric* serotype Paratyphi A have

been reported as the most common culture isolates from patients with febrile illnesses [14]. Globally, there are an estimated 22 million cases of *Salmonella enterica* serovar Typhi infection each year [9]. Typhoid and paratyphoid fever are common in developing countries, where millions of people are infected each year. Salmonella Typhi bacteria are estimated to cause between 10 million and 30million cases of typhoid in a year [15]. Typhoid is transmitted through contaminated food or beverages. The main method of transmission is by person to person spread either in hospital or in community. *S. Typhi* lives only in human and is carried in the bloodstream and intestinal tracts of people who have typhoid fever. Small number of those people who recover from typhoid fever continues to carry the bacteria and shed it in their stools, they are referred to as 'carriers'.

Still more to come, it is worthy to note that, malaria and typhoid fever still remain diseases of major public health importance in the tropics. Individuals in areas endemic for both diseases are at substantial risk of contracting both these diseases, either concurrently or an acute infection superimposed on a chronic one [16]. Malaria and typhoid fever usually present with similar symptoms particularly in their early stage (([26][17]). An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century and was named "typhomalarial fever" by the United States army [18]. This situation often presents a diagnostic problem and in some cases could lead to diagnostic confusion. Increasing multidrug resistance in Salmonella *enterica* serotype Typhi has been reported from various parts of the world [14].

II. Aim and objectives of the Study

This study is aimed at providing accurate and précised knowledge of the prevalence of *Plasmodium* and *Salmonella bacilli* as the evidence base data to be used in epidemiological studies for the eradication of Malaria and Typhoid fever in Nigeria and the world at large.

The objective of this study includes;

1. The utilization of diagnostic laboratory test results as the best tool for the treatment of Malaria and typhoid fever.
2. The revelation of the prevalence and demographic data of these diseases in relation to the patient's geographical location and base which aids in the allocation of disease control resources, strategies, drugs and professional manpower to the most endemic and highest risk prevalent zones.
3. The study of the prevalence and drug sensitivity of Malaria and Typhoid exposes the species with the highest prevalence and drug resistant strains creating room for new sensitive drug discovery for the disease eradication.

III. Materials and Method

3.1 Study Area/Location

The research was conducted in the University of Jos Clinic Laboratory (UHS Lab), located in Jos North Local Government Area of Plateau State, Nigeria. The Clinic serves the general medical needs of the University community (Staff, Students, and Staff dependants) and patients living in and around the University environment. The clinic also serves those that registered with the University Clinic as their Health Care Provider under the National Health Insurance Scheme (NHIS).

Study population:

A total of 200 patients (Male & Female) comprising of in- patients presenting at the clinic with signs and symptoms suggestive of malaria or typhoid fever. The clinical signs include; Fever, Headache, Nosiea, vomiting, diarrhoea, general body pain and weakness among other 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; they were apparently healthy individuals patronizing the clinic to obtain Medical certificates of fitness for admission into schools, job appointment, and marriage and for voluntary medical check-ups. Excluded from this study are those presently taking antimalarial and antibiotic drugs or that just finished these medications 4 to 5 days before visiting the clinic.

Ethical consideration:

The protocols 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 bio-data of the participants.

IV. Collection of specimen

1. Blood samples:

A total of five milliliter (5ml) of blood samples were aseptically collected by vein puncture. Two milliliter (2ml) were directly dispensed into the prepared brain heart infusion broth containing 18ml broth used for the blood culture,2ml dispensed into a plan tube used for the widal screening test and the remaining 1ml was dispensed into EDTA container.

2. Stool Sample:

The subjects were instructed on how to produce and submit the stool sample. The stool samples were collected into a clean wide mouth glass bottles container. They were submitted to the Laboratory within 2 hours of production.

Preparation of blood films:

1. Thick 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.
2. Thin blood films: The thin blood film was made by placing a drop measuring 2µl 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.

Staining of blood films:

1. **Thick blood films:** The dry thick blood films was stained using field stain A & B. The spread blood portion was covered first with field A for 2-3 minutes, washed off using tap water of Ph 7. This was replaced with field stain B and stained as above and air dried after which they were examined under the oil emersion objective of the microscope.
2. **Thin blood films:** The dry thin blood films were stained using Leishman stain. The slide was stained by covering the blood film with the stain for 2 minutes, double diluted with tap water Ph 7. After staining for 8 minutes, the slide was washed and the back wiped dry with cotton wool, and air dried after which they were examined under the oil emersion objective of the microscope [17]. This process was utilized in order to establish the plasmodium species recording the highest prevalence as reviled with microscopic peripheral thin blood films.

Stool culture:

Pea size of the stool sample 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 the work carried out by [27]. 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 ([26][27][17]).

Separation of serum:

The two milliliter of the blood sample 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 7^0C until when required for carrying out the Widal test.

V. Laboratory Analysis

Parasitological examination of slides for Malaria parasite test:

The thick films were stained using Field stain (A and B) while the thin films was stained using leishman stain. They were examined under the (x100) oil emersion power objective for the presence of any peripheral parasitaemia.

Serological Test for Typhoid fever screening:

The sera were screened for antibodies to *Salmonella* using the commercial antigen suspension reagents (Biosystem, febrile Serodiagnostics Barcelona, Spain EN ISO13485 and EN ISO 9001 Standards). A drop of the serum was placed on a tile, and it was then 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 titres of $\geq 1/80$.

Isolation of Salmonella:

Stool Culture:

With the aid of the applicator stick, a pea size of stool sample was emulsified into 5ml of Selenite F (SF) broth and incubated overnight at 37°C as described by ([25][22][19][17]).

After the overnight incubation, a loopful was sub cultured onto the *Salmonella-Shigella* Agar (SSA) and Deoxycholate Citrate agar (DCA) and incubated at 37°C for 24 hours. Suspected *Salmonella* colonies were identified using biochemical tests.

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, MaCconkey agar and Chocolate media and incubated aerobically at 37^0C for 24hrs. The brain heart infusion broth media were further incubated for another eight days and checked for microbial growth. Isolates were identified using biochemical tests.

Sensitivity Testing:

Pour plate method for sensitivity testing: The isolated Salmonella Species were inoculated into 10mls of peptone water and incubated at 37^0C for 2hrs. From this volume, enough quantity to cover the Mueller Hinton sensitivity agar was poured into the plate and excess drained off plate. The antibiotic discs were placed onto the plate and incubated aerobically at 37^0C for 24hrs. The zone of clearance were measured and recorded in millimeters.

Invitro Antimalarial and Antityphoid Drug Sensitivity Testing on Plasmodium and Salmonella Species

The antimalarial and antityphoid in-vitro drug sensitivity testing was carried out using a modified WHO Protocol as described by [19].

- These were done in accordance with the demonstration based on the principle, that the Plasmodium organism growing in RBC's could be cultured invitro [30]. Also in line with the works carried out by these researchers ([19][28][29]).

Briefly, the micro drug plates were pre- dosed with the antimalarial and anti-typhoid drugs as follows: - P-Alaxin: 5mg/5ml, Artemether lumefantrine: 5mg/5ml, Artesunate/Amodiaquine: 5mg/5ml and the stocked Anti typhoid drugs: ciprofloxacin 5mg/ml, cefuroxime 5mg/ml and Azithromycin 5mg/ml.

- A measured volume of 20 µl of anti malaria and anti typhoid drugs respectively was added into the well labeled Perspex trays.
- This was followed by the addition of 200 µl of the 2% McCoy's 5A medium, 20 µl of the parasitized RBC and 2 µl (1 drop) of AB+ Ag.
- The whole content was mixed and incubated at 37 under CO_2 candle jar.

The supporting McCoy 5A media was continuously added after every two hours. Incubation was stopped when greater than 40% of ring stage parasites had matured to mature schizont in the drug free control plates. The rate of turbidity in the wells containing the Salmonella species was checked and was used in the pour plate method for the antityphoid drug inhibition/sensitivity action.

Monitoring culture growth**:** After every 2hrs the medium was removed using a sterile Pasteur pipette without disturbing the cells. A drop of the culture content (Test samples) was placed on the slide and a thin and thick film. Fresh complete media (McCoy's 5A medium and 20% AB+ human serum) were added at the intervals of 2/2 hours into the plasmodium culture wells, mixed properly, and kept back in the incubator till the maturity time. Thin film was stained and examined for parasitaemia speciation and counts.

Smear Preparation and Staining: Three micro litters of the culture content were placed on a slide, at the center edge of the slide. The slides were then stained with Field A stain for 15 seconds and washed with water to remove excess stain, then counter stained with the stain B, for 15 seconds and washed with water to remove the excess stain. It was then dried and observed under the oil emersion of the microscope.

Finally, the thin blood films were stained with leishman stain, examined microscopically for parasitaemia at x100 objective. The results obtained were recorded and the presence or absence of malaria parasites seen compared with the initial parasitaemia films.

VI. Results

Table1; Prevalence and Distribution of *Malaria* Parasite (*Plasmodium* Species)

According to gender and age

Subjects within the age groups of 5 to 26years are at the highest risk of contracting malaria and typhoid since they are more prone to the hazardous environs.

Table 2; Prevalence of *Salmonella* Species with reference to isolates sensitivity to drugs according to age and gender

Table 1: Distribution of Malaria Parasitaemia in relation to age and sex

	Total	Male		Female		Total
Age group	No. examined	No. examined	No. (%) positive	No. examined	No. (%) positive	(%) positive
5 – 9	35	17	11(64.70)	18	13(72.22)	24
10 – 14	`31	15	13(86.66)	16	14(87.50)	27
15 – 19	25	12	10(83.30)	13	11(84.61)	21
20 – 24	24	12	8(66.66)	12	10(83.33)	18
25 – 29	17	7	5(71.42)	10	8(80.00)	13
30 – 34	15	5	3(60.00)	10	8(80.00)	11
35 – 39	20	``8	5(62.50)	12	10(83.33)	15
40 – 44	13	5	2(40.00)	8	6(75.00)	8
45 – 49	17	8	5(62.50)	9	7(77.77)	12
50 – 54	16	8	5(62.50)	8	5(62.50)	10
55 – 59	16	7	4(57.10)	9	6(66.66)	10
60+	21	10	7(70.00)	11	8(72.72)	15
Total	250	114	78(68.42)	136	108(77.94)	186

Key; Male = M; Female = F; MP Pos = No of Patients with positive MP; MP Neg =No of Patients with Negative MP; Total N = Total number.

Table 2: Prevalence of *Salmonella* Species with Reference to Isolates sensitivity to drugs According to Age and Gender

Age group	**Sal. Iso/Spp.**				**M**	**F**	**Control Group**	**Sal. Iso/Spp**			**M**	**F**
	ST	PA	B	C			ST	PA	PB	PC		
5-9	3	2	1	1	2	4	1	1	0	1	1	1
10-14	2	2	1	1	2	4	0	0	1	0	1	1
15-19	2	2	0	2	3	3	1	1	0	1	1	1
20-24	1	1	1	1	2	4	1	0	0	1	0	1
25-29	1	0	0	0	1	2	0	0	0	0	0	1
30-34	2	0	1	0	2	1	0	0	0	0	0	0
35-39	2	0	1	0	1	2	0	0	0	0	0	1
40-44	1	1	1	0	1	2	0	0	1	0	0	0
45-49	2	1	2	0	1	2	0	0	0	0	1	0
50-54	1	1	1	0	1	1	1	0	0	0	0	0
55-59	1	1	0	0	1	2	0	0	0	0	1	0
60+	1	1	1	1	1	2	1	0	0	0	0	1
Total	**19**	**12**	**10**	**6**	**18**	**29**	**5**	**2**	**2**	**3**	**5**	**7**

Salmonella Isolates = Sal Iso; Salmonella typhi = ST; Paraytphi A = PA; Paraytphi B = PB; Paraytphi C = PC

Sensitivity of the isolated *Salmonella* spp. indicating zones of inhibitions/clearance by the drugs

Infection	**Subjects (%) Plasmodium/Salmonella Preval.**	
	Febrile	**Control**
Positive for malaria parasite; invivo & invitro	39(19.5)	8(16.0)
Salmonella Isolate sensitive to *Cip. (2 – 4mm)	14(7.0)	4(8.0)
Salmonella Isolate sensitive to *Cef. (2 – 3mm)	10(5.0)	1(2.0)
Salmonella Isolate sensitive to *Azith. (2mm)	8(4.0)	1(2.0)
Salmonella Isolate of sensitivity less than 2mm	9(4.5)	2(4.o)
Synergistic effects of antimalarial/antityphoid drug	26(13.0)	7(14.0)
Total	200(100)	50(100)

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

VII. Discussion and Conclusion

Discussion

Several anti-biotic have shown promising antimalarial effects and have been useful for malarial chemotherapy. Based on the result of the Malaria and Typhoid prevalence obtained in this study, Ciprofloxacin, an anti-typhoid drug exhibited high chemotherapeutic synergistic curative effect in malarial eradication. Over the last decade, fluoroquinolone have emerged as the mainstay of therapy for enteric fever. At the same time, increasing incidence of *Plasmodium* infection with *Salmonellae* resistant to Nalidixic acid, which usually display decreased susceptibility to fluoroquinolone, which has raised considerable global concern [14]. Worldwide drug resistance constitutes an impediment to the control of malaria [5]. Based on the findings in this research study, it was discovered that the intake of resistance antimalarial and antityphoid drugs unknowingly by the infected patients constituted the major impediment towards the eradication of these disease. This has led to the need for constant surveillance and monitoring for changes in the sensitivity of malaria to the different anti malaria drugs being used in their treatment.

6.2 Conclusion;

The results obtained in this research study, indicate prominent antimalarial action of ciprofloxacin invitro and in-vivo particularly in combination with Artemether Lumefantrin. This supports further evaluation of ciprofloxacin as a potential combination drug for treatment of drug-resistance cases of malaria. Malaria and Typhoid are endemic in Nigeria as can be seen from the result obtained in this study. The prevalence of malaria is of highest incidence among the female children of age bracket 10-14(87.5%) followed by their male counterpart 10-14 (86.6%). This was strictly seconded by the teenager 15-19 years recording 84.61% and 83.3% from male and female respectively, but generally recording the highest infection among the age bracket of 16 – 26 years. The prevalence was also of high record among the middle age 40-54 years yielding 83.33% and above. Similarly, *Salmonella Typhi*, *Paratyphi* A and B also recorded high prevalence in the same age groups as seen in the result obtained in this study.

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