Medical and Pharmaceutical Journal

Volume 2 Issue 1

Med. Pharm. J. Original article

Prevalence of Concomitant Bacteria among malaria Patients attending Government Hospitals in Ondo State, South-West Nigeria

K. O. **Ajayi**[1]*; F. O. **Omoya** [1]; M. K. **Oladunmoye** [1]. and, B. O. **Oladejo** [1].

[1]Department of Microbiology, Federal University of Technology, Akure PMB 704, Akure, Nigeria

***Corresponding Author**: Kehinde Oluyemi **Ajayi**

Email ID: ooluyhemikehinde@yahoo.com

PhD Student at Federal University of Technology, Akure

DOI: 10.55940/medphar202316

Submitted: 13- Jan-2023

Accepted: 20- Mar-2023

Published:31-Mar-2023

ABSTRACT

Background: Patients suffering from severe malaria have an increased risk of co-occurring bacterial sepsis, which results in higher mortality rates than malaria alone. Concomitant bacterial infections can worsen malaria symptoms, leading to increased illness and death rates. This study aimed to investigate the relationship between malaria and concomitant bacteremia among patients in Ondo State, Nigeria.

Aim: The study's objective was to determine the prevalence of bacterial concomitant among malaria patients and assess the relationship between malaria and bacteremia in the study population.

Material and Methods: A cross-sectional study was conducted among feverish individuals in certain Ondo State government hospitals. One milliliter of blood was collected from malaria-positive patients, confirmed using microscopic examination of Giemsa-stained thick and thin blood films, and an Malaria rapid diagnostic tests (RDT) kit was cultured for bacterial enumeration. The collected data were statistically analyzed using Chi-square and correlation tests, with a *P* value <0.05 considered significant.

Result: The study found a concomitant bacteria prevalence of 19.95% (85/426), with a significant (P= <0.001, r = 0.00334) positive correlation between malaria and bacterial concomitant. Age was significantly (p = 0.072, df= 18, $\chi 2$ = 27.389) associated with bacterial concomitant, and the mean bacterial count was 9.71±1.481 cfu/ml x 107. Furthermore, level of education, marital status and tribe also significantly (p<0.05) contributed to the prevalence of concomitant bacteria.

Conclusion: Our research concludes that malaria may increase the risk of bacteremia, and a significant association between malaria and concomitant bacterial infection exists. However, the reasons for bacteria's ability to invade and thrive in the bloodstream vary. The study's findings suggest that interventions are needed to address the high prevalence of concomitant bacterial infections among malaria patients in the study area.

KEYWORDS: Febrile patients, Malaria, Bacteremia concomitant, Prevalence

INTRODUCTION

Nigeria is a country where various diseases have been reported, caused by a range of pathogens including Plasmodium falciparum parasite (malaria fever), Salmonella serovars (enteric fever), Lassa virus (Lassa fever), arbovirus (yellow fever), Mycobacterium tuberculosis infection, and COVID-19 [1-3]. Differential diagnosis of malaria from non-malarial febrile infections could help to prevent and correctly treat malaria and non-malaria febrile infection. The Plasmodium falciparum is the root cause of 99.7% malaria cases in Nigeria, which happened to retain the record for the highest malaria burden and fatality rate globally with 23% and 24% respectively [4].

Many microorganisms infects human (such as viruses, bacteria, protozoa, fungi, and helminths) frequently exist together [5-7]. Evidence points to malaria infections as increasing the chance of systematic bacterial infections, which may raise mortality rates in cases of malaria. Studies from Africa demonstrate that individuals in malaria-hostile zones experience increased susceptibility to bacteremia, owing to malaria [8,9] . Moreover, those in high malaria-prevalence zones routinely face other infectious agents, making concomitant infections a norm rather than an exception. [9,10].

For almost a century, it has been well-established that there is a correlation between malaria and an increased susceptibility to invasive bacterial infections [11]. This phenomenon has been demonstrated in various Sub-Saharan African settings [12]. In cases of P. falciparum malaria, non-typhoidal Salmonella species (NTS), other Gram-negative bacteria [13,14], Enterobacteria, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, and Haemophilus influenza [15,16] are commonly found co-infecting with malaria. Patients co-infected with these bacteria and malaria have higher mortality rates than those infected with malaria alone [8,17]. This association is particularly strong in severe malarial anemia cases [12]. Possible mechanisms that make individuals with malaria more susceptible to bacteremia include intestinal translocations of bacteria, increased gut permeability, immunosuppression due to immunoparesis, impairment of phagocytic cell complement consumption, and increased erythrophagocytosis [17].

Differentiating between bacterial co-infections and malaria is a challenge for clinicians in malaria cases and requires proper laboratory tests. Diagnosis is often based solely on symptoms because it can be difficult to distinguish between the two conditions [8]. As a result, physicians must prioritize tracking blood cultures of malaria patients to ensure timely and accurate treatment.

AIM

The study's objective was to determine the prevalence of bacterial concomitant among malaria patients and assess the relationship between malaria and bacteremia in the study population.

MATERIALS AND METHODS

Study Area and Sample size determination

This study was carried out in Ondo State, focused mainly on the out-patients presented with febrile illness in selected government hospitals between October, 2018 and August, 2019 the duration which covered both raining and dry season, in Ondo State, Nigeria. A total of 515 blood samples were collected from patients presented with febrile illness. The study populations were from 10 selected government hospitals randomly selected from government hospitals in Ondo State covering all the three senatorial districts (Figure 1). For the purpose of this study, febrile illness was characterized as having a recorded axillary temperature of ≥37.5°C or a self-reported history of fever within the preceding 48 hours.

Equation 1 from Kwenti et al. (2017) was used to determine the sample size for our cross-sectional descriptive study, utilizing Fisher's formula.

$$N = \frac{Z^2 \times p\,(1-p)}{e^2} \quad \text{…equation 1}$$

where;

Z = 1.96

p = prevalence of malaria in Nigeria (45% Rapid Diagnostic Test (RDT), 27% Microscopy) NMIS (2015)

e = error rate = 0.05

The sample size is thus calculated (using the highest prevalence of RDT 45% = 0.45) as;

$$N = \frac{1.96^2 \times 0.45\,(1-0.45)}{0.05^2} = \frac{3.8416 \times 0.2475}{0.0025} = 380 \text{ samples}$$

Therefore, the sample size for this study was stated to be ≥ 380 samples

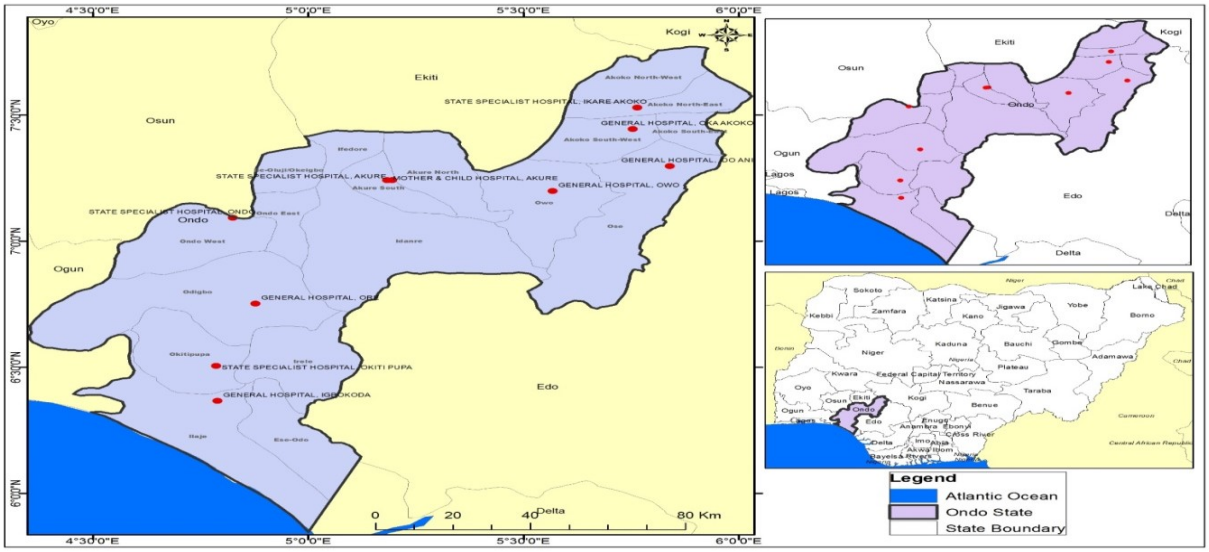

Figure 1: Location of the Sites of Sample Collection Represented on Ondo State Map Using the Geographic Coordinates

Selection and Enrollment of Participants

The study participants were outpatients attending government hospitals with symptoms of febrile illness, such as high body temperature (fever) and body pain. The inclusion criteria for participation were as follows: residency in the same area or near the study hospital, presence in the state during the onset of infection, informed consent, and current febrile symptoms. Children were required to weigh more than 6 kg and be aged between 6 months and 10 years old. Male, female, and pregnant women were included in the study. Initially, 741 volunteers participated, but only 515 individuals met the necessary requirements for participation, which included submitting a signed consent form, completing a questionnaire, and providing blood samples for examination. The age range of those who met the criteria was 5 to 84 years old.

Collection of Blood Samples from Patients

Sterile technique was used to collect 3 to 5 ml of whole blood from the consented patients, part of the blood was dispensed into sample tube that containing anticoagulant EDTA (Ethylene Diaminetetraacetic acid) following the method described by Cheesbrough [31]. To ensure the smooth collection of samples and avoid any complications, expert medical personnel, including Medical Laboratory Scientists and physicians, were responsible for the collection of blood samples in every hospital visited. All the blood samples were labeled correctly and processed within 1 hour of collection.

Blood Smear Preparation and Microscopy

Parasite estimation and parasite type identification were conducted utilizing a malaria blood smear microscopy technique. To prepare the thick and thin smears, about 6µl of EDTA whole blood was applied to the former and 3µl to the latter. We created thick film smears using fresh blood samples from three separate locations. The thin film was utilized to compare the atlas with the parasite seen in order to determine the species. Using 10% Giemsa solution, the thick blood films were given a good stain for 20 minutes and later bubbled with buffered distilled water (pH 7.2) (10 min post-soak). Absolute methanol was utilized to fix thin blood films instead of fixing while prestaining. After proper drying, the films were free from any wetness.

Microscopic examination for malaria parasites was performed by applying a drop of immersion oil onto the stained slide that had been allowed to dry. A 100x oil immersion objective lens was used in accordance with established procedures for detecting and identifying malaria parasites [31]. To ensure accuracy, two microbiology lab microscopists independently reviewed the slides, and any discrepancies (positive vs. The blood films were inspected for the presence of Plasmodium species. In cases of a difference in parasite density, indicated by

a ratio of >2 or a >Log10 difference, a third microscopist reviewed the slide. The observation of ring/trophozoite form of Plasmodium species confirmed a positive result. Negativism was also noted. Using the 100x objective lens, equation 2 was employed to calculate parasitaemia determined from thick blood film.

$$\text{Number of parasites/µL of blood} = \frac{8000 \times \text{Number of parasites counted against 100 WBC}}{100}$$

…. equation 2

Determination of Malaria using Rapid Diagnostic Test Kit

In accordance with the manufacturer's instructions, blood samples were subjected to malaria testing using the SD Bioline Pf kit (Standard Diagnostics, Hagal-Dong, Korea). This diagnostic test is recommended by the national malaria control program, and it is designed to detect the P. falciparum-specific histidine-rich protein-2 (PfHRP2) antigen. A 5.0 µL volume of whole blood was carefully applied to the sample pad of the test device, and a single drop of buffer was immediately added. The test was allowed to run for 3 minutes, during which time the blood migrated along the test strip by capillary action. A positive test result was indicated by the appearance of two red lines on the test strip, while a single red line indicated a negative result.

Preparation of Blood Samples for Isolation of Bacteria

The method for preparing blood samples was conducted as described by researchers [15]. After blood collection from patients, 2.0 mL of blood sample was dispensed gently from the syringe into a presterilized 2.0 mL Brain Heart Infusion broth (BHI) and mixed gently. The mixture of blood and BHI in the McCartney bottle was then transported to the research laboratory in the Department of Microbiology at Federal University of Technology, Akure for bacteriological examination.

The McCartney bottles that were confirmed positive for malaria via slides and RDT were incubated at 37 °C for 72 hours. After incubation, 100 µl of the bottle showing positive growth was cultured using a pour plate method on chocolate, mannitol salt, and MacConkey agar. The plates were incubated at 37 °C for 24 hours, and those with no growth were reexamined for growth after 48 hours of incubation.

RESULTS

Relationship between Malaria and Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

Relationship between malaria and concomitant bacteria among febrile malaria patients attending Government hospital in Ondo State is shown in Figure 2. The result showed that there was positive significant correlations (r^2 = 0.00334, P= <0.001) between the malaria and concomitant bacteria among febrile malaria patients. Of all 426(82.72%) patients that had malaria, 85(20.19%) were positive for bacteraemia and the association showed the liner graph plot which signified that the higher the parasite load, the higher the bacterial load. Parasites densities ranged between 209 and 22310 parasites/µl with a mean parasite density of 5522.17±183.30 parasites/µl,

while the bacterial counts ranged from 0 to 264.00 cfu/ml with the mean bacterial counts of 9.72±1.47 cfu/ml.

Gender based Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

The prevalence of concomitant bacteria among febrile malaria patients based on gender is shown in Figure 3. It was observed that among 426 (150 male; 276 female) malaria patients tested, the prevalence of concomitant bacteria in female was 48/276(17.39%) among which 42/276(15.225), 3/276(1.09%) and 3/276(1.09) had bacterial load of 1-100, 101-200 and >200 cfu/ml respectively while male was 37/150(24.67%) among which 32/150(21.33%) 4/150(2.67%) 1/150(0.67%) had bacterial load of 1-100, 101-200 and >200 cfu/ml respectively. Statistically, there was no significant ($p = 0.222$, $df= 3$, $\chi^2 = 4.394$) difference in gender based prevalence of concomitant bacteria among malaria patients. Also, the mean bacterial counts of female and male were 8.51±1.74 and 11.94±2.73 cfu/ml respectively and there was no significant ($p<0.05$) difference in the mean bacterial counts.

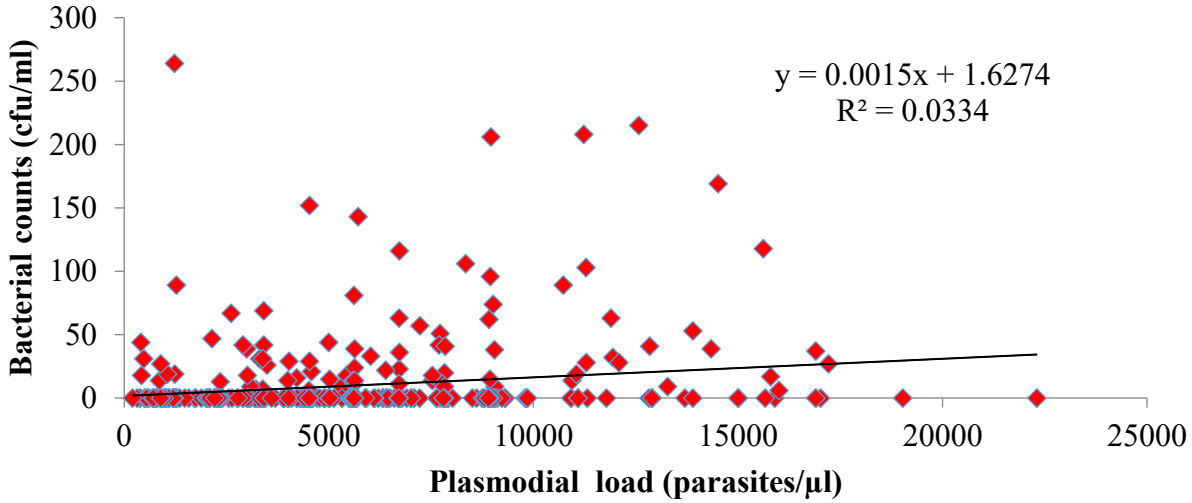

Figure 2: Relationship between Malaria and Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

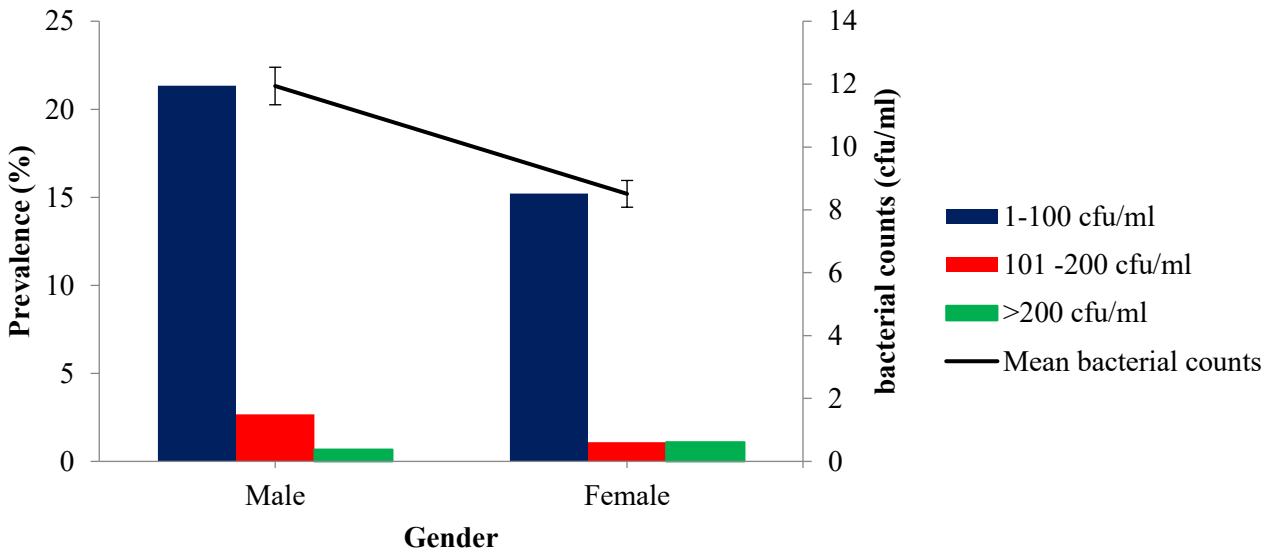

Figure 3: Gender base Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.222$, $df = 3$, $\chi^2 = 4.394$)

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Age range

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo State based on age range (Figure 4). The prevalence of concomitant bacteria among different age groups 0 – 10(9), 11 – 20(52), 21 – 30(60), 31 – 40(84), 41 – 50(72), 51 – 60(81), and >60(68) years were 0%, 9/52(17.31%), 8/60(13.33%), 14/84(16.67%), 27/72(37.50%), 13/81(16.04%) and 14/68(20.59%) respectively. Among the patients in age range 11 – 20 years, 7(13.46%) and 2(3.85%) had bacterial counts of 1-100 and 101 – 200 cfu/ml respectively, 31 – 40 years, 13(15.48%) and 1(1.19%) had bacterial counts of 1-100 and 101 – 200 cfu/ml respectively, 41 – 50 years, 23(31.94%) 2(2.78%) and 2(2.78%) had bacterial counts of 1-100, 101 – 200 and >200 cfu/ml respectively, 51 – 60 years, 12(14.81%) and 1(1.23%) had bacterial counts of 1-100 and 101 – 200 cfu/ml respectively and >60 years, 11(16.18%), 1(1.47%) and 2(2.94%) had bacterial counts of 1-100, 101 – 200 and >200 cfu/ml respectively. However, statistically, there was no significant ($p = 0.072$, $df = 18$, $\chi^2 = 27.389$) relationship between age groups and prevalence of concomitant bacteria. Also, the mean bacterial counts of 0 – 10, 11 – 20, 21 – 30, 31 – 40, 41 – 50, 51 – 60 and >60 years were 0.00±0.00, 7.42±3.17, 4.18±1.78, 5.58±2.31, 20.90±5.47, 7.88±2.45 and 13.10±4.91 cfu/ml respectively, the mean

bacterial counts of age range 41 – 50 was significantly ($p<0.05$) higher than others.

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Level of Education

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo state based on level of education is shown in Figure 5. The result revealed that among the patients with no formal education (39), primary (27), secondary (106) and tertiary (254) level of education the prevalence of concomitant bacteria were 5/39(12.82%), 5/27(18.52%), 28/106(26.42%) and 47/254(18.50%) respectively. Among those with secondary level of education, 20/106(18.87%), 6/106(5.66%) and 2/106(1.89%) had bacterial counts of 1 – 100, 101 – 200 and >200 cfu/ml respectively. Statistically, there was significant ($p = 0.047$, $df= 9$, $\chi^2 = 17.101$) relationship between concomitant bacteria and level of education. Also, the mean bacterial counts were 4.69±2.51 (no formal education), 5.37± 2.50 (primary), 17.33± 4.39 (secondary) and 7.78±1.55 (tertiary), the mean bacterial counts of those with secondary level of education were significantly ($p<0.05$) higher than others.

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Occupation

Figure 6 shows the prevalence of concomitant bacteria among febrile malaria patients attending the Government hospital in Ondo state, in relation to occupation. The prevalence of concomitant bacteria among civil servants (77), entrepreneurs (74), farmers (47), pensioners (32), students (70), traders (65), and unemployed individuals (61) were 19/77 (24.68%), 21/74 (28.38%), 10/47 (21.28%), 7/32 (21.88%), 11/70 (15.71%), 10/65 (15.38%), and 7/61 (11.48%), respectively. The proportion of those who had bacterial counts greater than 100 cfu/ml was less than 0.5% in all occupations.

Statistically, there was no significant relationship ($p = 0.560$, $df= 18$, $\chi2 = 16.471$) between concomitant bacteria and occupation among the malaria patients. Additionally, the mean bacterial counts were 12.71 ± 3.62 (civil servants), 9.66 ± 2.69 (entrepreneurs), 13.94 ± 6.62 (farmers), 12.72 ± 7.02 (pensioners), 7.09 ± 2.67 (students), 8.92 ± 3.84 (traders), and 5.05 ± 2.99 (unemployed individuals) cfu/ml. There was no significant difference ($p < 0.05$) between the mean bacterial counts.

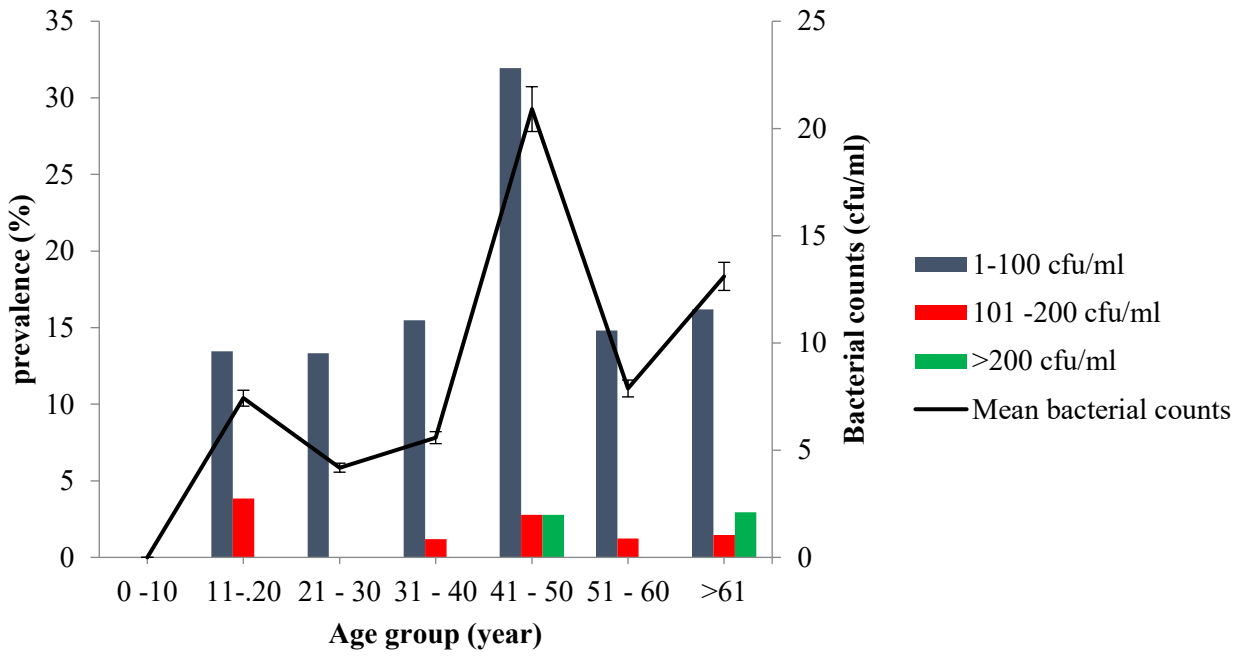

Figure 4: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Age range

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.072$, $df = 18$, $\chi^2 = 27.389$)

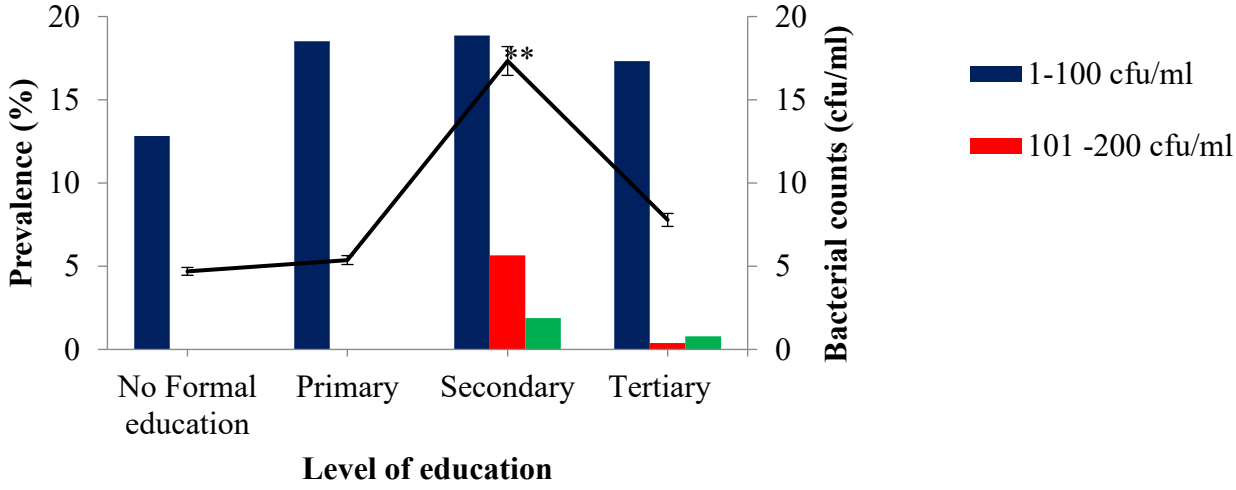

Figure 5: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Level of Education

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.047$, $df = 9$, $\chi^2 = 17.101$)

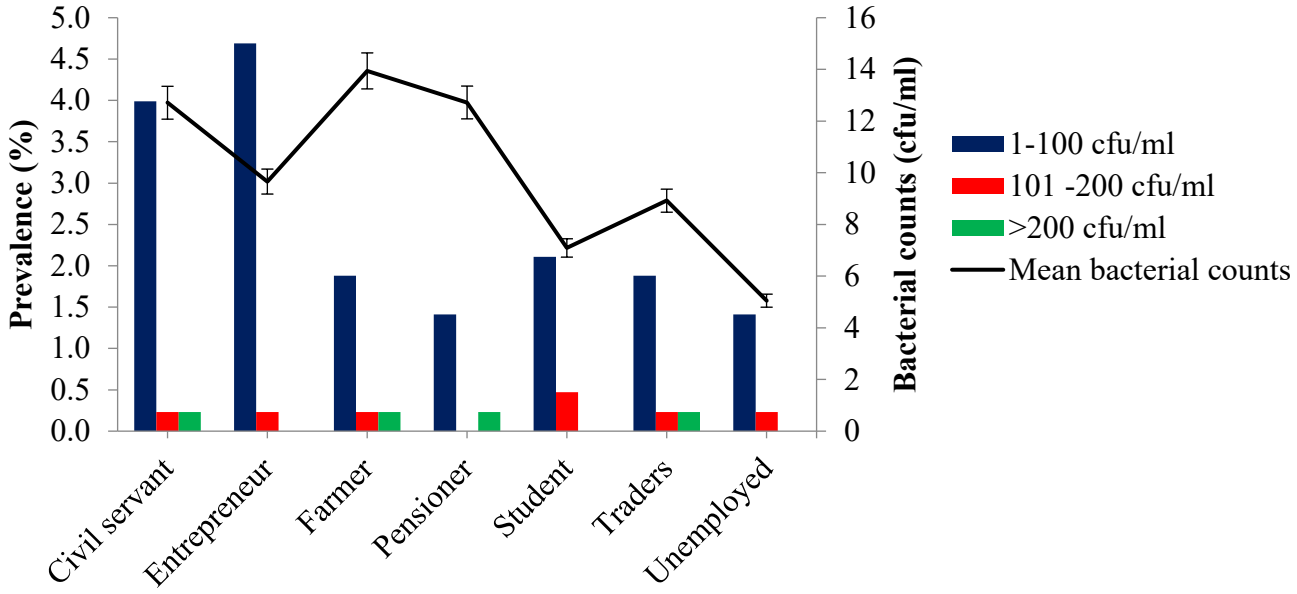

Figure 6: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Occupation

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.560$, $df = 18$, $\chi^2 = 16.471$)

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Tribe

The prevalence of concomitant bacteria among febrile malaria patients attending a government hospital in Ondo State in relation to tribe is shown in Figure 7. The results revealed that the prevalence of concomitant bacteria among the Yoruba (401), Hausa (7), and Igbo (18) tribes were 78/401 (19.45%), 3/7 (42.89%), and 4/18 (22.22%), respectively. Among the Yoruba tribe, 17.21% (69), 1.25% (5), and 1.00% (4) had bacterial counts of 1-100, 101-200, and >200 cfu/ml, respectively, while among the Igbo, 11.11% (2) had bacterial counts of 1-100 and 101-200 cfu/ml. Statistically, there was a significant ($p = 0.029$, $df = 6$, $\chi2 = 14.080$) relationship between tribe and concomitant bacteria among malaria patients. Also, the mean bacterial counts of the Yoruba, Hausa, and Igbo tribes were 9.26±1.49, 10.29±6.52, and 19.61±10.47 cfu/ml, respectively, and there was no significant ($p<0.05$) difference between the bacterial counts.

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Religion

The prevalence of concomitant bacteria among febrile malaria patients attending a government hospital in Ondo state in

relation to religion is revealed in Figure 8. The prevalence of concomitant bacteria among Christian (360), Muslim (63), and other religions (3) were 70/360 (19.44%), 14/63 (22.22%), and 1/3 (33.33%), respectively. Among the Christians, 59/360 (16.39%), 7/360 (1.94%), and 4/360 (1.11%) had bacterial counts of 1-100, 101-200, and >200 cfu/ml, respectively. Statistically, there was no significant ($p = 0.727$, $df= 6$, $\chi2 = 3.629$) relationship between concomitant bacteria and religion among malaria patients. Also, the mean bacterial counts of Christians, Muslims, and other religions were 10.05±1.68, 8.02±2.53, and 6.00±1.00 cfu/ml, respectively, and there was no significant ($p<0.05$) difference between the counts.

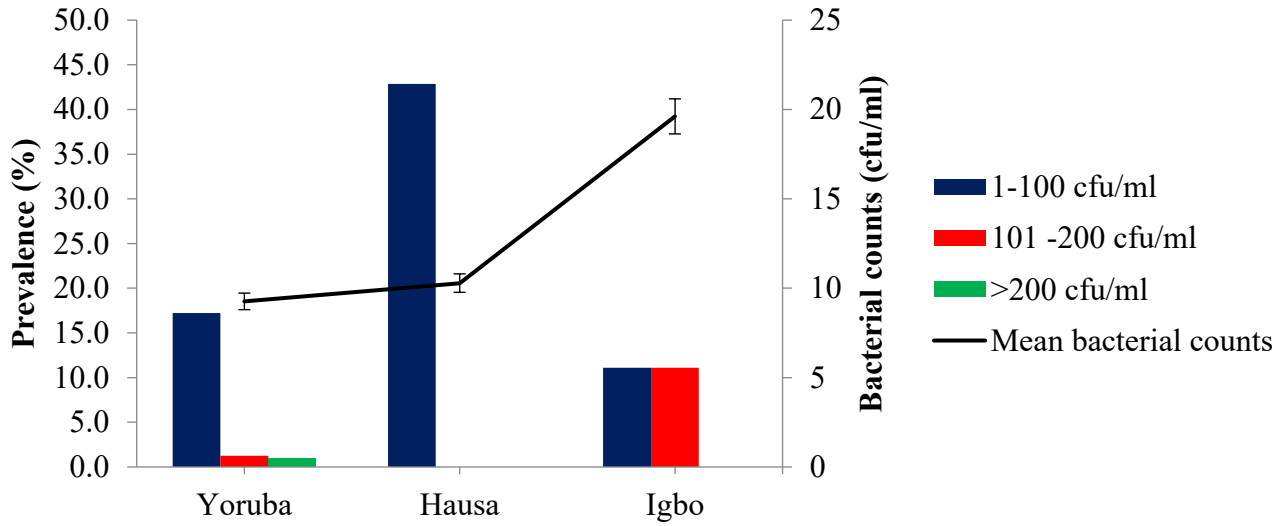

Figure 7: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Tribe

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.029$, $df= 6$, $\chi^2 = 14.080$)

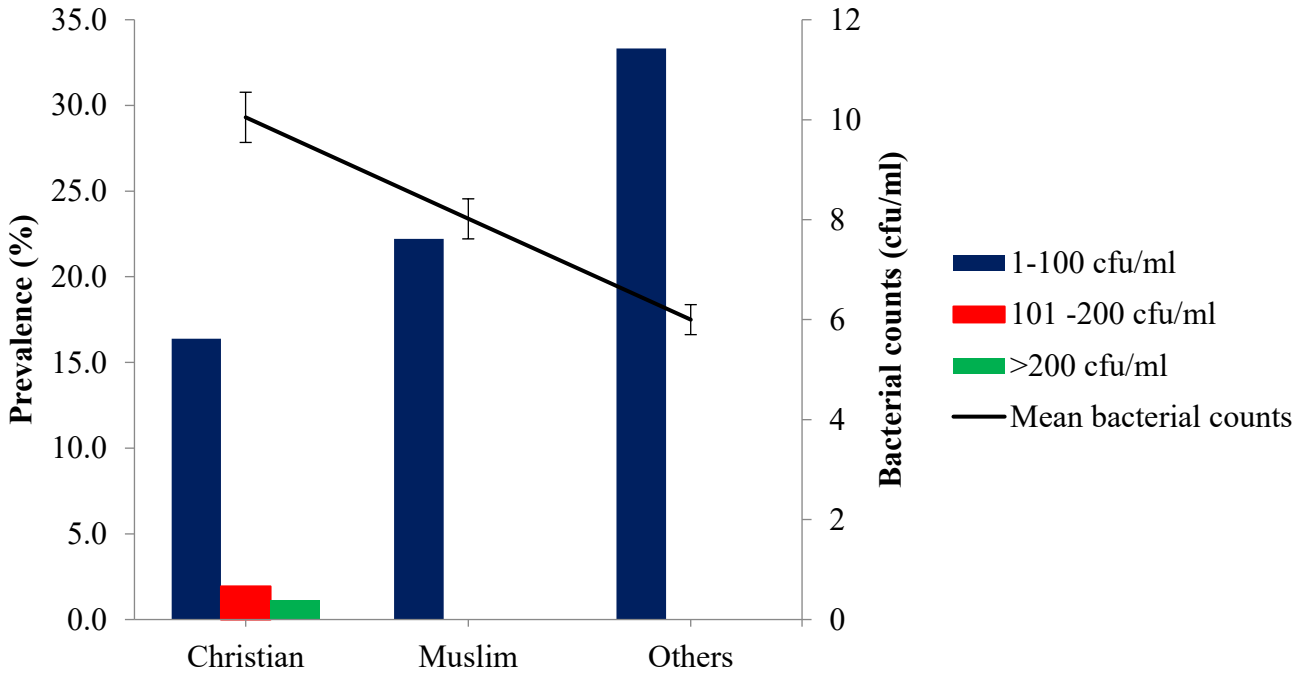

Figure 8: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Religion

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.727$, $df = 6$, $\chi^2 = 3.629$)

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Marital Status

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo state in relation to marital status is revealed in Figure 9. The prevalence of concomitant bacteria in single (109), married (267), divorced (25), widowed (17) and widower (8) were 14/109(12.84%), 56/267(20.97%), 5/25(20.00), 7/17(41.18%) and 3/8(37.5%) respectively. Among the singles, 12/109 (11.01%) and 2/109(1.83%) had bacterial load of 1-100 and 101-200 cfu/ml respectively, married with bacterial load of 1-100, 101-200 and >200 were 50/267(18.73%), 4/267(1.50%) and 2/267(0.75%) respectively while 5/17(29.41%) and 2/17(11.76%) of the widowed had bacterial load of 1-100 and >200 cfu/ml respectively. Also, among the widower, 2/8(25%) and 1/8(12.5%) had bacterial load of 1-100 and 101-200 cfu/ml respectively. Statistically, there was significant ($p = <0.001$, $df = 12$, $\chi^2 = 35.737$) relationship between concomitant bacteria and marital status among malaria patients. The mean bacterial counts were 5.55±1.84 (single), 9.83±1.85 (married), 9.20±4.62 (divorced), 29.00±16.78 (widowed) and 23.38±13.46 (widower) cfu/ml and the bacterial counts of the

widowed was significantly ($p<0.05$) higher than others.

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of commercial antimalarial drug

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo State based on the use of commercial antimalarial drug is shown in Figure 10. The prevalence of concomitant bacteria was higher (78/389(20.05%) among those that have taken antimalarial drugs than those that have not (7/37 (18.92%) before visiting the hospital. Also, among those that have taken antimalarial drug, the bacterial load range were 17.22% (1-100 cfu/ml), 1.80% (101-200 cfu/ml) and 1.03% (>200). However, statistically, there was no significant ($p = 0.775$, df= 3, $\chi^2 = 1.108$) relationship between used of antimalarial drug before visiting hospital and concomitant bacteria among the malaria patients. The mean bacteria counts of those that have taken antimalarial drug and those that have not were 9.93±1.58 and 7.51±3.00 cfu/ml respectively and there was no significant ($p<0.05$) difference between the counts.

Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of herbs

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo State based on the use of herbs is shown in Figure 11. The prevalence of concomitant bacteria was higher (57/283(20.14%) among those that have not taken herbs than those that have taken herbs (28/142 (19.72%) before visiting the hospital. Also, among those that have taken herbs, the bacterial load range were 15.49% (1-100 cfu/ml), 3.52% (101-200 cfu/ml) and 0.7% (>200) while those that have not taken herbs their bacterial load range were 18.37% (1-100 cfu/ml), 0.71% (101-200 cfu/ml) and 1.06% (>200). However, statistically, there was no significant ($p = 0.163$, df= 3, $\chi^2 = 5.128$) relationship between used of herbs before visiting hospital and concomitant bacteria among the malaria patients. The mean bacteria counts of those that have taken herbs and those that have not were 11.08±2.66 and 9.07±1.77 cfu/ml respectively and there was no significant ($p<0.05$) difference between the counts.

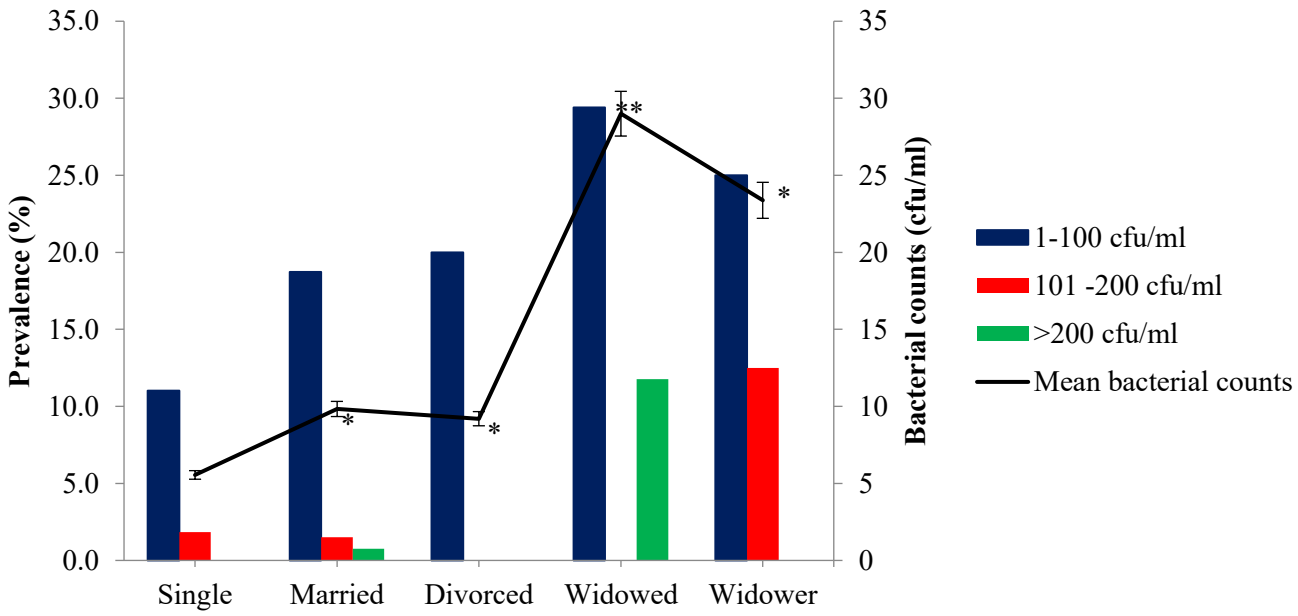

Figure 9: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Marital Status ($p = <0.001$, $df = 12$, $\chi^2 = 35.737$)

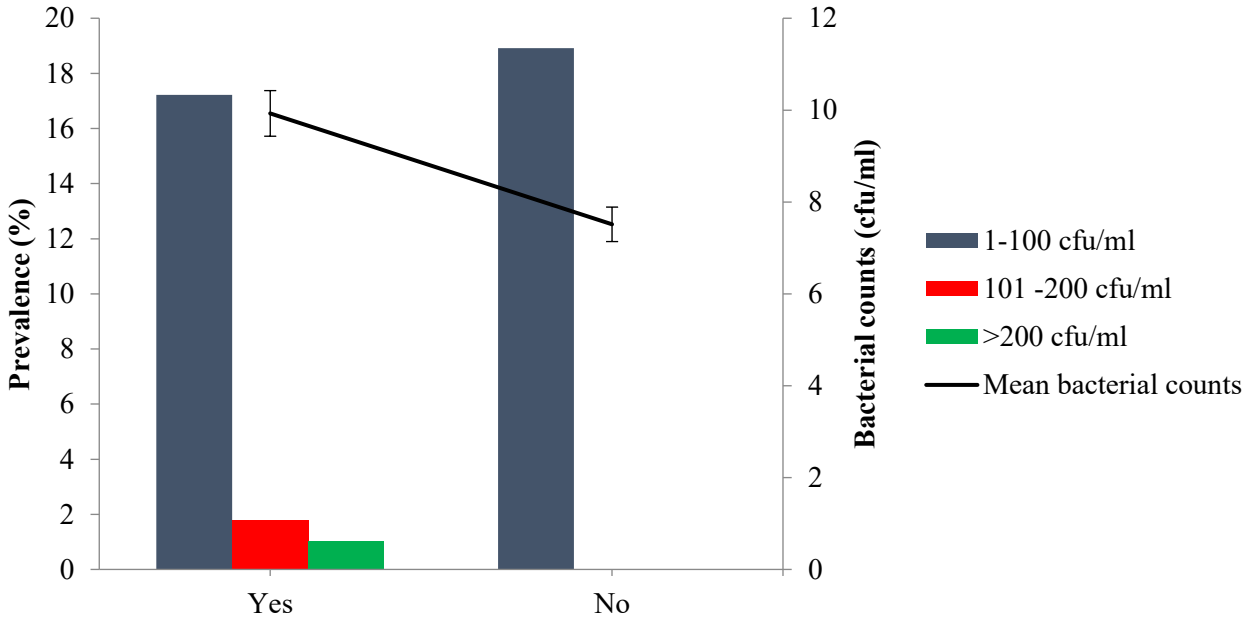

Figure 10: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of Commercial antimalarial drug ($p = 0.775$, $df = 3$, $\chi^2 = 1.108$)

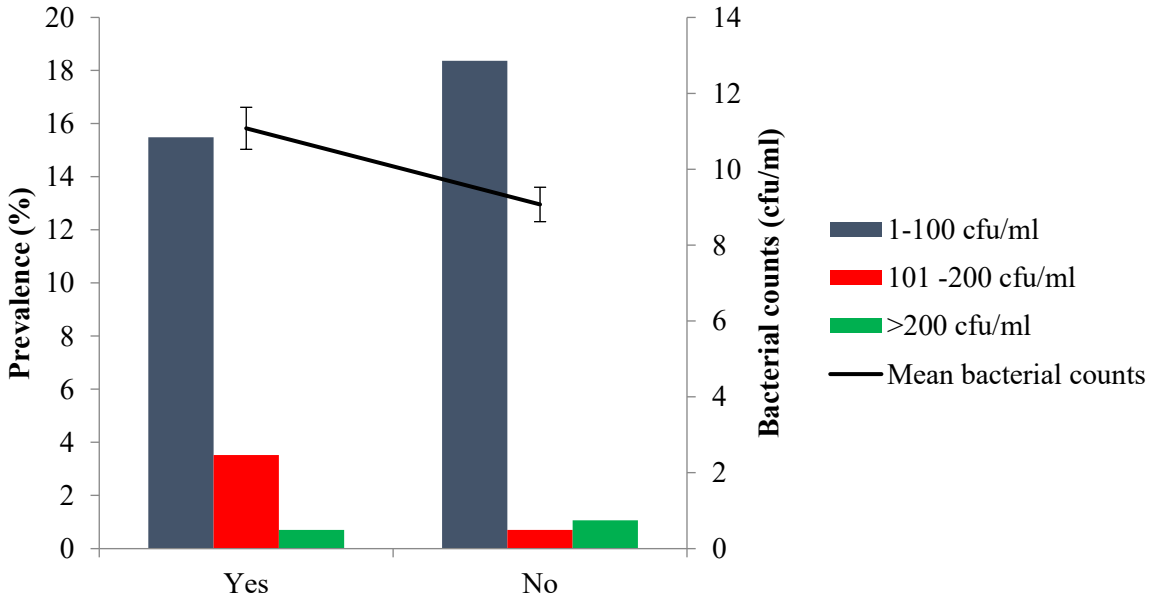

Figure 11: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of Herbs

The prevalence of data is expressed in percentage form. It's worth noting that trends represented as 'p' are deemed significant if they're at or below 0.05. ($p = 0.163$, $df = 3$, $\chi^2 = 5.128$)

Age Distribution of Polymicrobial Bacteremia among Malaria Patients Attending Government Hospitals in Ondo State

The distribution of polymicrobial bacteriaemia among malaria patients attending government hospital in Akure is shown in Table 1. It was noted that 76.47% (65/85) of the patients had unimicrobial bacteriaemia while 12.94% and 10.59% of the patients had polymicrobial bacteriaemia of 2 and more than 2. Also, the concomitant bacterial was higher among the age range 41 to 50 years (31.76%) followed by 31 to 40 (16.47%). Statistically, there was a significant ($p<0.001$, df= 15, $\chi^2 = 50.14$) association between age and poly-microbial bacteriaemia.

Table 1: Age Distribution of Polymicrobial Bacteremia among Malaria Patients Attending Government Hospitals in Ondo State

Isolates	Unimicrobial Bacteremia	Polymicrobial bacteremia (2)	Polymicrobial bacteremia (>2)	Total (%)
0 -10	0	0	0	0(0.00)
11 – 20	4	2	3	9(10.59)
21 – 30	7	1	0	8(9.41)
31 – 40	12	2	0	14(16.47)
41 – 50	24	3	0	27(31.76)
51 – 60	10	1	2	13(15.29)
61 above	8	2	4	14(16.47)
Total (%)	65(76.47)	11(12.94)	9(10.59)	85

$p<0.001$ (df= 15, $\chi^2 = 50.14$)

DISCUSSION

Clinical management of malaria patients necessitates addressing any concurrent bacterial infections which could provide valuable insights into the pathogenesis of co-infection. In this study. Of all 426 patients that had malaria, 85(20.19%) were positive for bacteraemia and a strong positive correlation existed between the two. The report of malaria prevalence among febrile patience in the study area was in our previous study [4]. Previous studies in Nigeria reported that there was no association between malaria and concomitant bacteria [8,19] and Kenya [20]. The significant association reported in this study may be due to the differences in the study population as the previous studies examined children and other out patients but this study only focused on the febrile population. Also, time and region of study may have accounted for the differences.

Several sub-Saharan countries have recorded an increase in systemic bacterial infections with high associated mortality rates due to recent or current malaria infections [8]. Infecting the body with one microorganism can create a hospitable environment for other pathogenic microorganisms to flourish. Take, for

example, the impact of P. falciparum on the innate immune system's various cells (including dendritic cells, macrophages, and neutrophils), as well as the alteration of the adaptive immune system and B cell population. In addition, certain regulatory cytokines that malaria unleashes to control the pro-inflammatory response could potentially impede the mucosal immune response against invasive bacteria, which could ultimately result in bloodstream infection [21].

The prevalence of malaria coinfection found in this study (20.19%), was lower than what was reported in Venezuela (34.2%) [22], India (60%) [23] and corroborate the study reported in Brazil (20%) [24]. However the prevalence of bacterial infection among malaia patients have been reported in different parts of Nigeria and are; Ebonyi (21.2%), Ibadan (16.7%), Kaduna State (36.6%), Akoko (73.9%), Lagos (27.6%), Benin (39%), Imo State (42%) and Sokoto (10.3%) [25]. The differences in reported studies and this study might be due to seasonal variation of sampling, difference in geographical locations, type of patients used as only febrile patients were included for this study and type of organisms considered for the co-infection. As a precaution, doctors must maintain a level of suspicion for coinfection in instances of atypical manifestations or inadequate treatment response in malaria cases.

The bacterial counts ranged from 0 to 264.00 cfu/ml with the mean bacterial counts of 9.72±1.47 cfu/ml. In light of the growing trends of bloodstream bacterial infections globally [26], Outlined in this study is the need to conduct pathogen load investigations. Baseline studies are needed due to limited published data on bacterial loads within bloodstream infections in the country. It is crucial to tackle this issue.

The prevalence of concomitant bacteria and mean bacterial load were higher in male than female but not statistically significant and larger proportion of both gender had bacterial load of less than 100 cfu/ml. This is contrary to the findings of Birhanie *et al.* [25] in Ethiopia and Oluyege *et al.* in Ekiti [8] , Nigeria who reported higher prevalence of malaria and concomitant bacteria among female than male. However, other study in Sokoto, Nigeria reported higher prevalence among male than female [26]**.** The higher prevalence of concomitant bacteria in male as observed in this study could be due to different factors such as presence of virulent gene in bacteria to invade the host blood stream [8], host immunological status and social status.

The socio-demographic characteristics of the patients were compared with prevalence and load of concomitant bacteria among febrile patients that are malaria positive, it was noted that there was higher prevalence of concomitant bacteria and mean bacterial counts among the age group 51-60 years than other age groups, concomitant bacteria was more prevalent among those with secondary level of education and they have higher mean bacterial load than others. Also, based on the occupation, concomitant bacteria was more prevalent among the entrepreneur

while the bacterial load was higher among farmers than others, tribe, concomitant bacteria was more prevalent among the Igbos while the bacterial load was higher among the Hausas than others, religion, concomitant bacteria was more prevalent among those with other religion other than Christianity and Muslim while the bacterial load was higher among the Christians than others and marital status, concomitant bacteria was more prevalent among the widowed as well as the bacterial load than others. Although there is paucity of information on this relationship among febrile people with malaria therefore this findings will serve as baseline study for prevalence of concomitant bacteria and the bacterial load in relationship with socio-demographic characteristics. Patients with coinfections were found to be at a higher risk of experiencing complications, indicating that concurrent infection with another pathogen could worsen the clinical progression of malaria. [22]. Furthermore, statistically, there was a significant ($p<0.05$) relationship between level of education, tribe, marital status and concomitant bacteria prevalence in this study, this could serve as guide in epidemiological study and control of concomitant bacteria in malaria.

The prevalence of concomitant bacteria and bacteria load were higher among those that have taken antimalarial drugs than those that have not before visiting the hospital. Among patients who had not consumed herbs prior to their hospital visit, a greater occurrence of coexisting bacteria and bacterial levels was discovered compared to those who had used herbs. The specific antimalarial medication used by these individuals was not a factor in the study's findings. Nevertheless, it is crucial to promote education that discourages the utilization of non-recommended drugs for malaria treatment, as emphasized by Mabbott et al. [7], and since antimalarial drug cannot inhibit the growth of bacteria this could have led to the increase in bacteria load. moreover, It is known that the utilization of herbs has the capacity to treat bacterial infections. However, this study revealed a more significant occurrence of such infections amongst herb users, which may have stemmed from variances in the herbs they used. Furthermore, while some herbs have been documented to merely suppress microorganisms, there exists a lack of scientific accreditation regarding their effectiveness as treatment, including an absence of established dosages [29]. Another factor that could have contributed to the high prevalence rate for herb users in this particular study is the harvesting time and processing method used, as suggested by Oladunmoye and Kehinde [28]. The higher prevalence and bacterial load among those that have taken either antimalarial or herbs before visiting the hospital could also be as a result of delayed treatment by physician because of self-medication.

It was noted that some patients had polymicrobial bacteriaemia which has significant association with age. Though the species of bacteria were not determined but this could increase the complication of

malaria infection. Some limitations exist in this study, given that only febrile patients were enrolled. In order to assess the true burden of coinfections in malaria, future studies should focus on asymptomatic individuals. Additionally, the virulence gene of the bacteria was not explored, with the possible presence of certain virulence genes potentially accounting for bacteria's ability to invade and survive in the blood, rather than just malaria.

CONCLUSION

In conclusion, this study provides a fundamental understanding of the prevalence of bacterial co-infection with malaria among febrile populations in Ondo State. The results confirm the co-occurrence of bacteremia and malaria, showing a significant correlation between the two. The findings suggest that malaria may increase the susceptibility of children with fever to bacteremia in the studied area. Additionally, level of education, tribe, and marital status were found to be significantly associated with bacterial co-infection with malaria. Further research should be conducted in other study locations to investigate other potential risk factors of malaria and bacterial co-infection.

RECOMMENDATIONS

Improved diagnostic, therapeutic, and preventive approaches are urgently needed for bacterial co-infection with malaria. The antimicrobial resistance patterns of these bacteria should be studied to aid effective treatment. Delay in either diagnosis or initiation of therapy for these infections could lead to fatal outcomes, hence self-medication with drugs or herbal products should be discouraged in the study area.

Ethical Approval

The Helsinki Declaration (WMA, 2001) was observed by the research team in securing ethical approval from Ondo State Health Research ethics committee (OSHREC), while ensuring participants' confidentiality and right to voluntary participation. The children's parents/guardians were given written informed consent which included the freedom to withdraw from the study without prejudice.

The study objectives and methods were explained to each of the participants prior to administration of questionnaire or interviews which were conducted in both English and Yoruba languages. All the information obtained was treated with utmost confidentiality and used for the research purposes only.

Competing interests

None

Funding

None

REFERENCES

1. Plaza JJ, Hulak N, Zhumadilov Z, Akilzhanova A. Fever as an important resource for infectious diseases research. Intractable & rare diseases research. 2016;5(2):97-102.
2. Fatiregun AA, Isere E, Dosumu M, Agunbiade O, Onyibe R. Lassa fever awareness and knowledge among community residents in

Ondo State, Nigeria. Journal of community medicine and primary health care. 2019;31(2):26-35.

3. Omoya F, Ajayi K. Prevalence of malaria among febrile patients attending government hospitals in Ondo State, South-West Nigeria. American Journal of Epidemiology & Public Health. 2020;4(4):17-24.

4. Malaria Report Sheet for 2019. WHO's World Malaria factsheet. World Health Organization. 2020;7–9.

5. Rigaud T, Perrot-Minnot MJ, Brown MJ. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society B: Biological Sciences. 2010;277(1701):3693-702.

6. Brogden KA, Guthmiller JM, Taylor CE. Human polymicrobial infections. The Lancet. 2005;365(9455):253-5.

7. Mabbott NA. The influence of parasite infections on host immunity to co-infection with other pathogens. Frontiers in immunology. 2018 Nov 8;9:2579. DOI: 10.3389/fimmu.2018.02579

8. Oluyege A, Dada K, Ojo-Bola O. Evaluation of the bacterial profile and pathogenicity in concomitant bacteremia with malaria among children in Ekiti state. Int J Trop Dis Health. 2017;21(1):1–7. DOI: 10.9734/ijtdh/2017/28313

9. Scott JA, Berkley JA, Mwangi I, Ochola L, Uyoga S, Macharia A, Ndila C, Lowe BS, Mwarumba S, Bauni E, Marsh K. Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. The Lancet. 2011;378(9799):1316-23.

10. Berkley JA, Maitland K, Mwangi I, Ngetsa C, Mwarumba S, Lowe BS, Newton CR, Marsh K, Scott JA, English M. Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. Bmj. 2005;330(7498):995.

11. Dondorp A, Nosten F, Stepniewska K, Day N, White N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet (London, England). 2005 Aug 1;366(9487):717-25.

12. Medana IM, Day NP, Sachanonta N, Mai NT, Dondorp AM, Pongponratn E, Hien TT, White NJ, Turner GD. Coma in fatal adult human malaria is not caused by cerebral oedema. Malaria journal. 2011;10(1):1-4.

13. Bronzan RN, Taylor TE, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, Njobvu A, Kondowe W, Chalira C, Walsh AL, Phiri A. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. The Journal of infectious diseases. 2007;195(6):895-904.

14. Bassat Q, Guinovart C, Sigaúque B, Mandomando I, Aide P, Sacarlal J, Nhampossa T, Bardají A, Morais L, Machevo S, Letang E. Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital. Tropical Medicine & International Health. 2009 Sep;14(9):1011-9.

15. Mtove G, Amos B, Nadjm B, Hendriksen IC, Dondorp AM, Mwambuli A, Kim DR, Ochiai RL, Clemens JD, Von Seidlein L, Reyburn H. Decreasing incidence of severe malaria and community-acquired bacteraemia among hospitalized children in Muheza, north-eastern Tanzania, 2006-2010. Malaria journal. 2011;10(1):1-1.

16. Maltha J, Guiraud I, Kaboré B, Lompo P, Ley B, Bottieau E, Van Geet C, Tinto H, Jacobs J. Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. PLoS One. 2014;9(2):e89103.

17. Haldar K, Murphy SC, Milner Jr DA, Taylor TE. Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. Annu. Rev. Pathol. Mech. Dis.. 2007 Feb 28;2:217-49.

18. Essuman VA, Ntim-Amponsah CT, Astrup BS, Adjei GO, Kurtzhals JA, Ndanu TA, Goka B. Retinopathy in severe malaria in Ghanaian children-overlap between fundus changes in cerebral and non-cerebral malaria. Malaria Journal. 2010;9(1):1-6.

19. Egbe CA, EnabulElE OI. Aetiologic agents of fevers of unknown origin among patients in Benin City, Nigeria. The Malaysian Journal of Medical Sciences: MJMS. 2014;21(1):37.
20. Were T, Davenport GC, Hittner JB, Ouma C, Vulule JM, Ong'Echa JM, Perkins DJ. Bacteremia in Kenyan children presenting with malaria. Journal of clinical microbiology. 2011;49(2):671-6.
21. Koganti R, Yadavalli T, Naqvi RA, Shukla D, Naqvi AR. Pathobiology and treatment of viral keratitis. Experimental eye research. 2021 Apr 1;205:108483.
22. Forero-Peña DA, de Mora MS, Rodríguez ID, Gamardo ÁF, Chavero M, Figuera L, Camejo-Avila NA, Marcano MV, Hidalgo M, Arenas CJ, Arévalo-Herrera M. High prevalence of viral and bacterial coinfections in malaria in Venezuela.
23. Mandage R, Kaur C, Pramanik A, Kumar V, Kodan P, Singh A, Saha S, Pandey S, Wig N, Pandey RM, Soneja M. Association of dengue virus and leptospira co-infections with malaria severity. Emerging Infectious Diseases. 2020;26(8):1645.
24. Magalhães BM, Siqueira AM, Alexandre MA, Souza MS, Gimaque JB, Bastos MS, Figueiredo RM, Melo GC, Lacerda MV, Mourão MP. P. vivax malaria and dengue fever co-infection: a cross-sectional study in the Brazilian Amazon. PLoS neglected tropical diseases. 2014;8(10):e3239.
25. Birhanie M, Tessema B, Ferede G, Endris M, Enawgaw B. Malaria, typhoid fever, and their coinfection among febrile patients at a rural health center in Northwest Ethiopia: a cross-sectional study. Advances in medicine. 2014;2014.
26. Maharath A, Ahmed MS. Bacterial Etiology of Bloodstream Infections and Antimicrobial Resistance Patterns from a Tertiary Care Hospital in Malé, Maldives. International Journal of Microbiology. 2021;2021.
27. Alhassan HM, Shidali NN, Manga SB, Abdullahi K, Hamid KM. Co-infection profile of Salmonella typhi and malaria parasite in Sokoto-Nigeria. Global Journal of Science, Engineering and Technology. 2012;2(201):13-20.
28. Oladunmoye MK, Kehinde FY. Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. African Journal of Microbiology Research. 2011;5(19):2991-3004.
29. Lagnika L, Djehoue R, Yedomonhan H, Sanni A. Ethnobotanical survey of medicinal plants used in malaria management in South Benin. Journal of Medicinal Plants Research. 2016;10(41):748-56.
30. Kwenti TE, Kwenti TDB, Njunda LA, Latz A, Tufon KA, Nkuo-Akenji T. Identification of the Plasmodium species in clinical samples from children residing in five epidemiological strata of malaria in Cameroon. Trop Med Health. 2017;45(1):14. DOI: 10.1186/s41182-017-0058-5
31. Cheesbrough M. District Laboratory Practice in Tropical Countries. Vol. 50. Cambridge, UK.: Cambridge University Press; 2014
32. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Nurs Ethics. 2002;9(1):105–9. DOI: 10.1191/0969733002ne486xx

How to cite this article:
Ajayi , K. O.; Omoya, F. O. ; Oladunmoye, M. K.. and, Oladejo; B. O.., P. Prevalence of Concomitant Bacteria among malaria patients attending Government Hospitals in Ondo State, South-West Nigeria. Med. Pharm. J. 2022;1(1):44- 64.

Available from: http://pharmacoj.com/ojs/index.php/Medph/article/view/16