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

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Abstract

Patients with severe malaria are at increased risk of developing concomitant bacterial sepsis. Bacteremia and malaria co-infection have a higher case fatality compared to those with malaria infection only. The existence of concomitant bacterial infections can further complicate the manifestation of malaria and increase the morbidity and mortality. Therefore this study was carried out to determine the relationship between bacteremia concomitant among malaria patients in Ondo State, Nigeria. A cross sectional study was conducted among febrile patients in selected government Hospitals in Ondo State. One milliliter of blood of patients confirmed to be malaria positive with microscopic examination of Giemsa stained thick and thin blood films and RDT kit was cultured for enumeration of bacterial concomitant. All data obtained were statistically analyzed using Chi-square and correlation test with a *P* value <0.05 considered significant. The study showed 19.95% (85/426) concomitant bacteria prevalence and a significant (P= <0.001, r = 0.00334) positive correlation between malaria and concomitant bacteria, also there was significant (p = 0.072, df= 18, χ^2 = 27.389) association between age and concomitant bacteria while the mean bacterial counts was 9.71±1.481 cfu/ml x 10^7. Also, level of education, marital status and tribe contributed significantly (p<0.05) to the prevalence of concomitant bacteria. In conclusion, the results show that there is a significant association between bacteremia and malaria in the study area and malaria could predispose to bacteremia, however, the ability of the bacteria to invade and survive in the blood is due to several factors.

Key words: febrile patients, malaria, bacteremia concomitant, prevalence

Introduction

Fever caused by *Plasmodium falciparum* parasite (malaria fever), *Salmonella* serovars (enteric fever), Lassa virus (Lassa fever), arbovirus (yellow fever), *Mycobacterium tuberculosis* infection and COVID-19 have been reported in Nigeria (Elhadad *et al.,* 2015; Plaza *et al.,* 2016; Fatiregun *et al.,* 2019; Omoya and Ajayi, 2020). Differential diagnosis of malaria from non-malarial febrile infections could help to prevent and correctly treat malaria and non-malaria febrile infection. Nigeria accounted for the highest global malaria burden (23%), malarial death (24%) and 99.7% of the malaria cases are caused by *Plasmodium falciparum* (WHO, 2020).

Many parasites that infect humans (e.g., viruses, bacteria, protozoa, fungal and helminths parasites) often co-occur within individuals (Brogden *et al.,* 2005; Rigaud *et al.,* 2010; Mabbott, 2018). There is strong evidence that recent or current infection with malaria increases the risk of systemic bacterial infections which could increase mortality rates in malaria (Oluyege *et al.,* 2017). Studies conducted in Africa suggest that malaria has been shown to strongly predispose people in areas of malaria endemicity to bacteremia (Scott *et al.,* 2011; Oluyege *et al.,* 2017), in addition, people living in areas with high malaria prevalence are frequently exposed to other infectious agents rendering co-infections as a rule rather than an exception (Berkley e*t al.,* 2005; Scott e*t al.,* 2011).

An association between malaria and susceptibility to invasive bacterial infection has been known for almost a century (Dondorp *et al.,* 2005a), and has been repeatedly documented in different settings across Sub-Saharan Africa (Medana *et al*. 2018). The most frequent pathogens associated with *P. falciparum* malaria are nontyphoid *Salmonella* species (NTS), other Gram-negative bacteria (Bronzan *et al.,* 2007; Bassat *et al.,* 2009), Enterobacteria, *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Haemophilus influenza* (Mtove *et al.,* 2011; Maltha *et al.,* 2014; Oluyege *et al.,* 2017). There co-occurrence during malaria infection increase the case fatality compared to those with malaria infection only (Haldar *et al.,* 2007; Oluyege *et al.,* 2017). Other studies have suggested that the association is particularly strong in the case of severe malarial anemia (Dorovini *et al.,* 2011; Medana *et al*. 2018).

Intestinal translocations of bacteria or increased gut permeability, immunosuppression through immunoparesis impairment of phagocytic cell complement consumption and increased erythrophagocytosis have been suggested as possible mechanisms that predisposes individuals with malaria to bacteraemia (Graham *et al.,* 2000; Essuman *et al.,* 2010).

It is difficult clinically to differentiate malaria and bacterial co-infections without appropriate laboratory investigations. In most cases of malaria and bacterial co-infections, diagnoses are made on the basis of clinical symptoms and treatment is presumptive without laboratory confirmation (Oluyege *et al.,* 2017). Therefore, blood culture surveillance among malaria infected is crucial for clinicians to ensure a timely and appropriate management response.

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). Febrile illness was defined in this study as those with axillary temperature ≥37.5 °C or reported history of fever in the past 48 hours.

The sample size was determined using standard epidemiological formula (Fisher's formula for cross-sectional descriptive study) as follows; (Kwenti *et al.*, 2017) in equation 1.

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

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

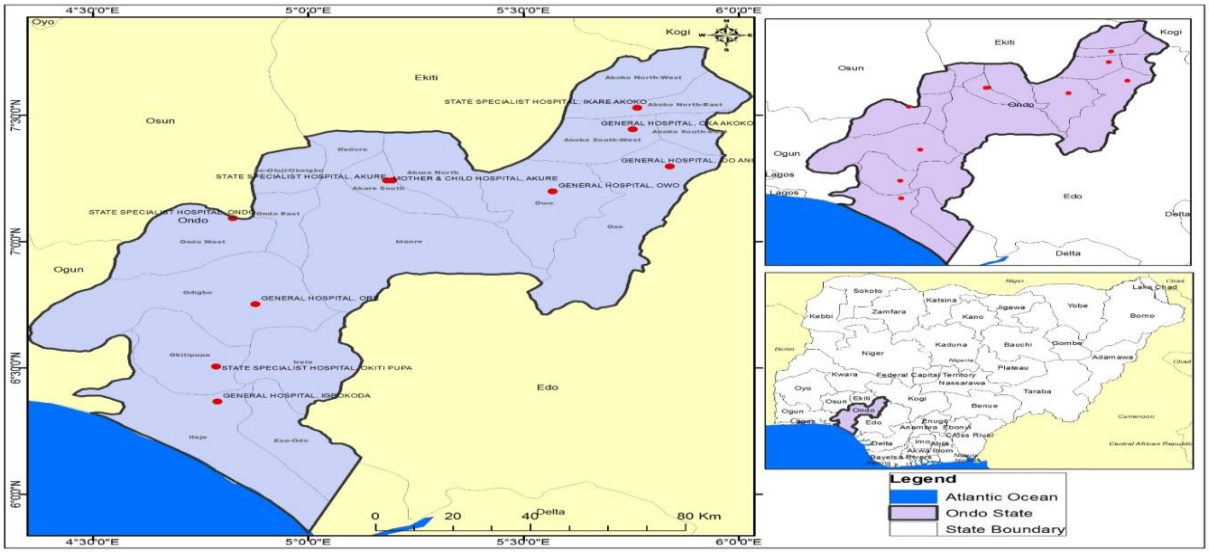

Figure 1: Location of the Sites of Sample Collection Represented on Ondo State Map Using the Geographic Coordinates

Ethical consideration and informed consent

The ethical approval was issued by Ondo State Health Research ethics committee (OSHREC). A written informed consent was obtained from the participants, parents/guardians of the children.

Individuals were assured of voluntary participation, confidentiality of their test results and opportunity to withdraw at any time without prejudice, in line with the Helsinki Declaration (WMA, 2001). 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.

Selection and Enrollment of Participants

Participants were out patient attending government hospital, presenting with symptoms of febrile illness such as high body temperature (fever) and body pain. Inclusion criteria for participants were: residency within 60 km of the study clinic, must have been in the state during the onset of the infection, informed consent and present febrile symptoms. Those considered children were between 6 months and 10 years of age and above 6kg in weight. Male, female and pregnant women were considered for the study. A total of 741 individuals volunteered to participate in this study; however, 515 individuals, aged 5 to 84 years, met the inclusion criteria (written signed consent, completed questionnaire, and blood samples for examination).

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 bottle containing EDTA (Ethylene Diaminetetraacetic acid) as anticoagulant following the method described by Cheesbrough (2014). In order to avert complications during collection of samples, all blood samples were collected by professionals; Medical Laboratory Scientist and physician working in each of the hospitals visited. All the blood samples were labeled correctly and processed within 1 hour of collection.

Blood Smear Preparation and Microscopy

Blood smear microscopy method was used for malaria parasite, about 6µl of EDTA whole blood was used for thick smear and 3µl for thin smear preparation. The thick smear was used for parasite estimation and thin film was used to determine the type of parasite by comparing the parasite seen with atlas. Thick film smears were made on three spots from fresh blood samples. The films were properly dried (without prestaining fixing). Thin blood films were fixed with absolute methanol and later stained along with thick blood films using 10% Giemsa solution for 20 minutes and subsequently washed (after 10 min) using buffered distilled water (pH 7.2).

A drop of immersion oil was applied on the dried stained slide and examined microscopically for malaria parasites using 100x oil immersion objective lens. The films were examined following standard procedure for the detection and identification of malaria parasites (Cheesbrough, 2014). For control measure, slides were read by two independent microscopist in microbiology laboratory, and in the case of any disparity (positive vs. negative, different *Plasmodium* species, difference in parasite density >Log10 or ratio >2 in case of parasite density ≤400/µL and >400/ µL respectively) they were read by a third microscopist. Slides were considered positive when ring / trophozoite form of *Plasmodium* species was observed in the blood film.

Parasitaemia was determined in thick blood film using the100x objective lens and calculated as stated in equation 2,

Number of parasites/µL of blood=

$$\frac{8000 \times \text{Number of parasites counted against 100 WBC}}{100} \quad \text{…. equation 2}$$

Determination of Malaria using Rapid Diagnostic Test Kit

The malaria rapid diagnostic test (RDT) recommended by the national malaria control program, SD Bioline Pf (Standard Diagnostics, Hagal-Dong, Korea) detecting *P. falciparum*-specific histidine-rich protein-2 (PfHRP2), was performed on EDTA blood samples according to the manufacturers' instructions. A 5.0 µL of whole blood was gently applied to the sample point of the kit and immediately followed by addition of a drop of buffer, the blood was left for 3 minutes to allow the flow from the sample application point to the end of the strip. The presence of double red lines indicate a positive result while a single line was red as negative.

Preparation of Blood Samples for Isolation of Bacteria

Blood samples were prepared as described in the method of (Maltha *et al.*, 2014) Immediately after the collection of blood from patients, two (2.0 mL) milliliter of blood sample was dispensed gently from the syringe into presterilised 2.0 mL of Brain Heart Infusion broth (BHI) and gently mixed, the mixture of blood and BHI in McCartney bottle was transported to research laboratory, Department of Microbiology, Federal University of Technology, Akure for bacteriological examinations.

The mixture of blood and BHI in McCartney bottle that the slides and RDT have been confirmed to be positive for malaria were incubated at 37 ^{o}C for 72 hours after which 100 µl of the bottle that showed positive growth was cultured using pour plate method on chocolate, manitol salt and MacConkey agar and incubated at 37 ^{o}C for 24 hours, the plates that showed 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(15.22), 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, χ^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.

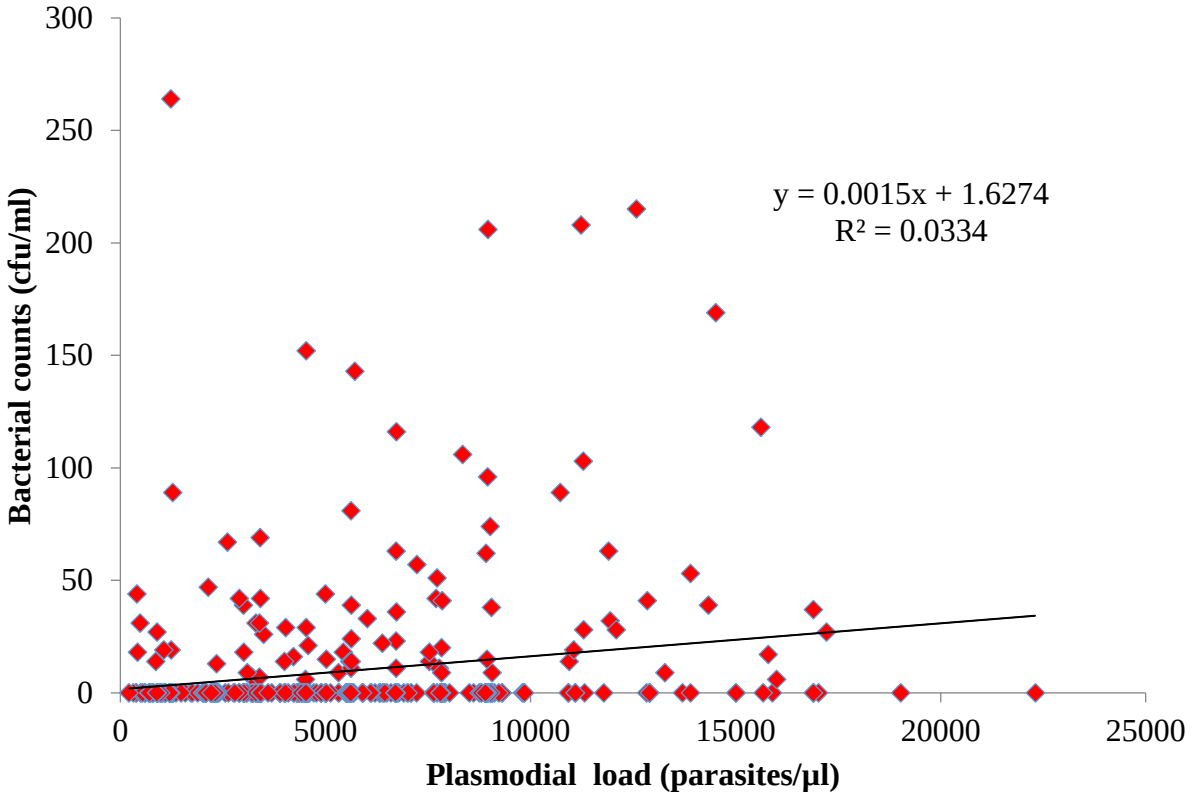

Figure 2: Relationship between Malaria and Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

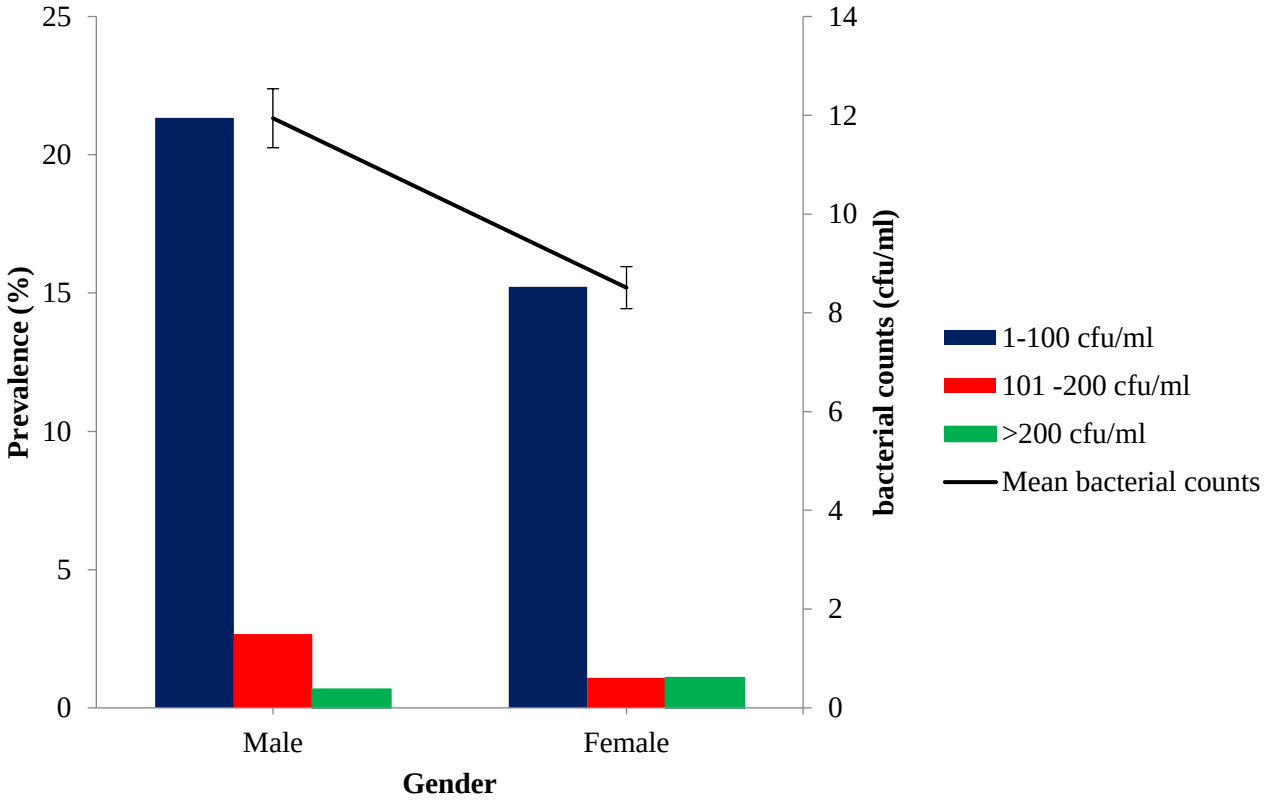

Figure 3: Gender base Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State

Data is presented as percentage prevalence. *p* for trends is significant at 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

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo state in relation to occupation is shown in Figure 6. The prevalence of concomitant bacterial among the civil servant (77), entrepreneur (74), famer (47), pensioner (32), student (70), traders (65) and unemployed (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 that had bacterial counts greater than 100 cfu/ml were less than 0.5% in all the occupations. Statistically, there was no significant ($p = 0.560$, $df= 18$, $\chi^2 = 16.471$) relationship between concomitant bacteria and occupation among the malaria patients. Also, the mean bacterial counts were 12.71±3.62 (civil servant), 9.66±2.69 (entrepreneur), 13.94±6.62 (famer), 12.72±7.02 (pensioner), 7.09±2.67 (student), 8.92±3.84 (trader) and 5.05±2.99 (unemployed) cfu/ml and there was no significant ($p<0.05$) difference between the mean bacterial counts.

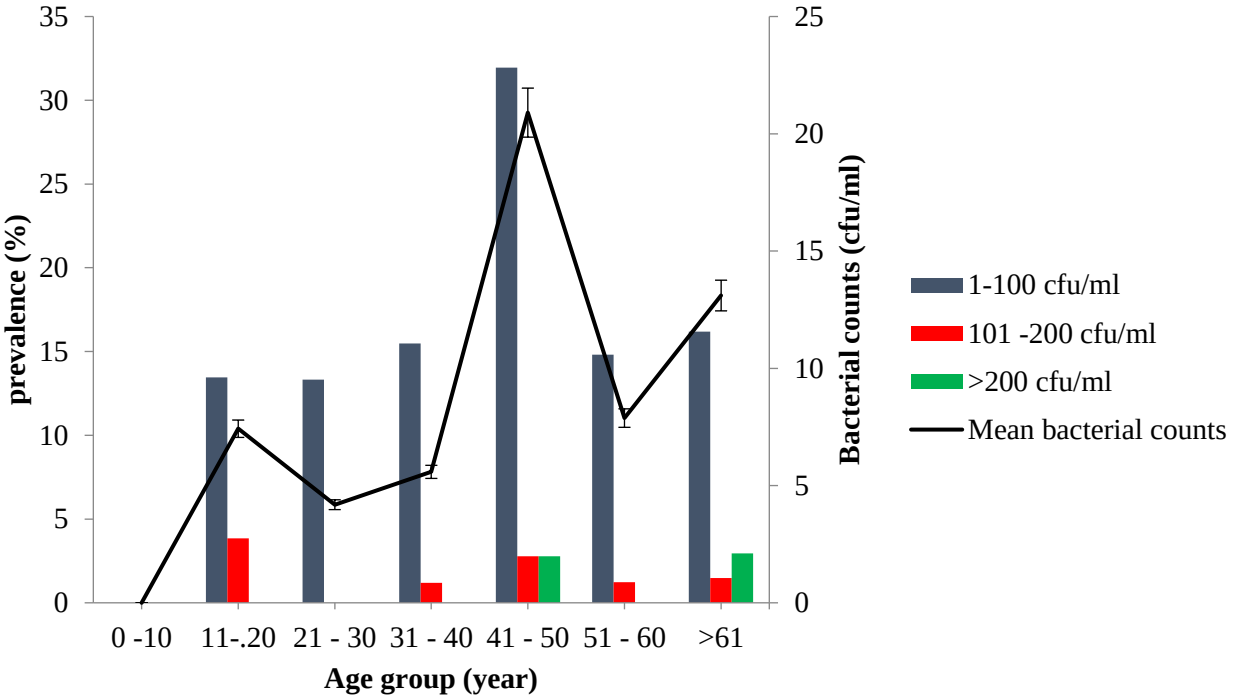

Figure 4: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Age range

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. ($p = 0.072$, df= 18, $\chi^2 = 27.389$)

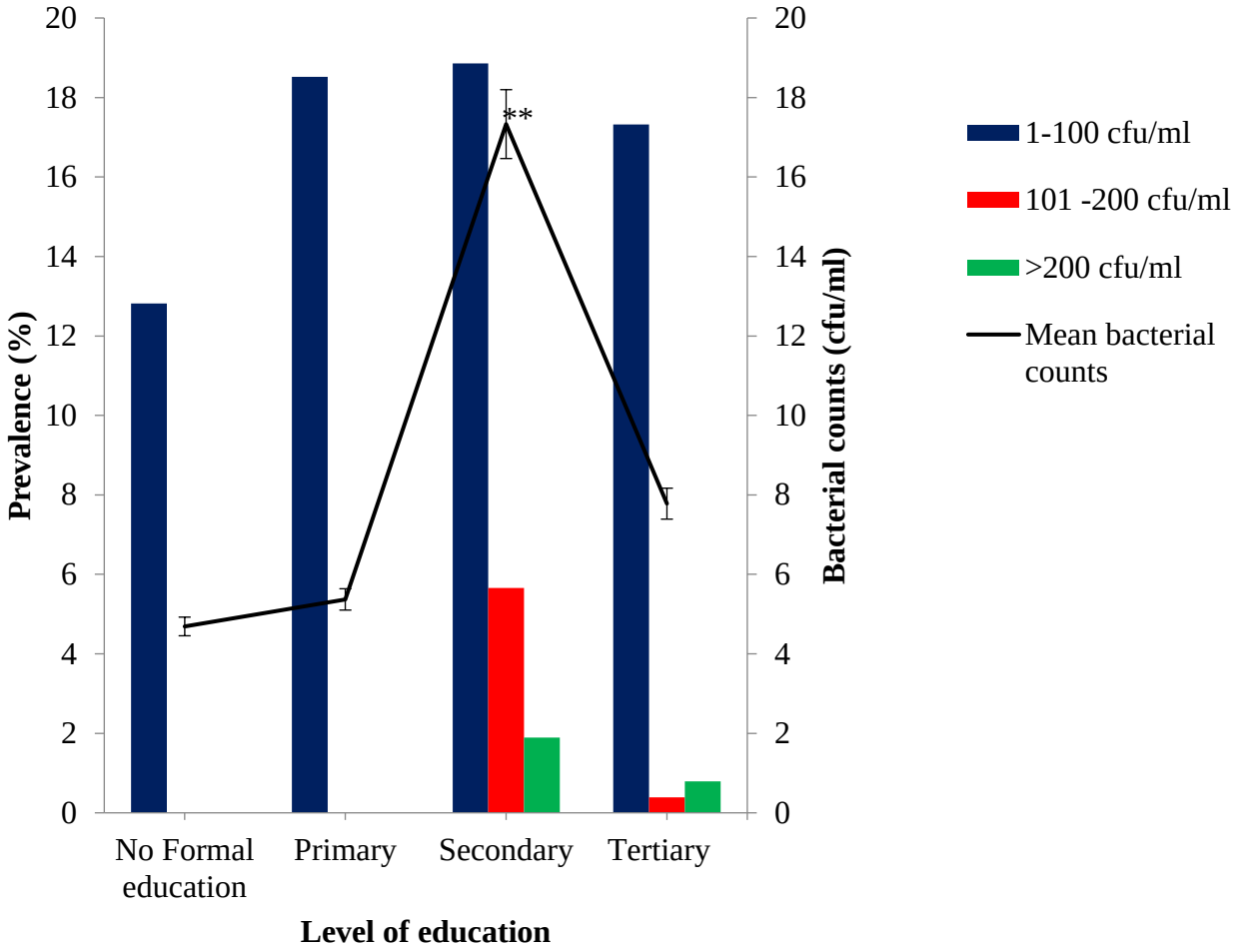

Figure 5: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on Level of Education

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. (p = 0.047, df= 9, χ^2 = 17.101)

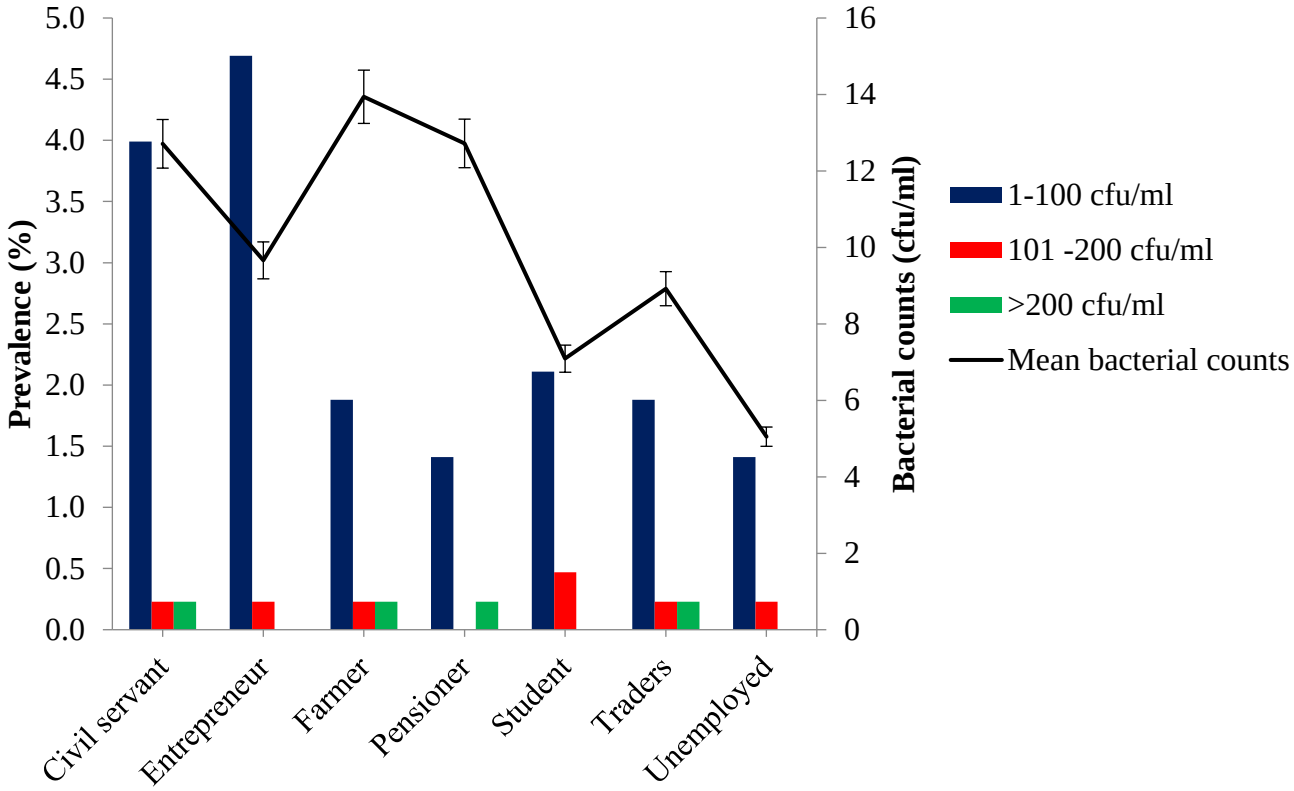

Figure 6: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Occupation

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. (p = 0.560, df= 18, χ^2 = 16.471)

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

Prevalence of concomitant bacteria among febrile malaria patients attending Government hospital in Ondo state in relation to tribe is shown in Figure 7. The result revealed that the prevalence of concomitant bacteria among 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, χ^2 = 14.080) relationship between tribe and concomitant bacteria among malaria patients. Also, the mean bacterial counts of 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

Prevalence of concomitant bacteria among febrile malaria patients attending 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 Christian, 59/360(16.39%), 7/360(1.94%) and 4/360(1.11%) had the bacterial counts of 1-100, 101-200 and >200 cfu/ml respectively.

Statistically, there was no significant (p = 0.727, df= 6, χ^2 = 3.629) relationship between concomitant bacteria and religion among malaria patients. Also, the mean bacterial counts of Christian, Muslim and other religions were 10.05±1.68, 8.02±2.53 and 6.00±1.00 cfu/ml and there was no significant (p<0.05) difference between the counts.

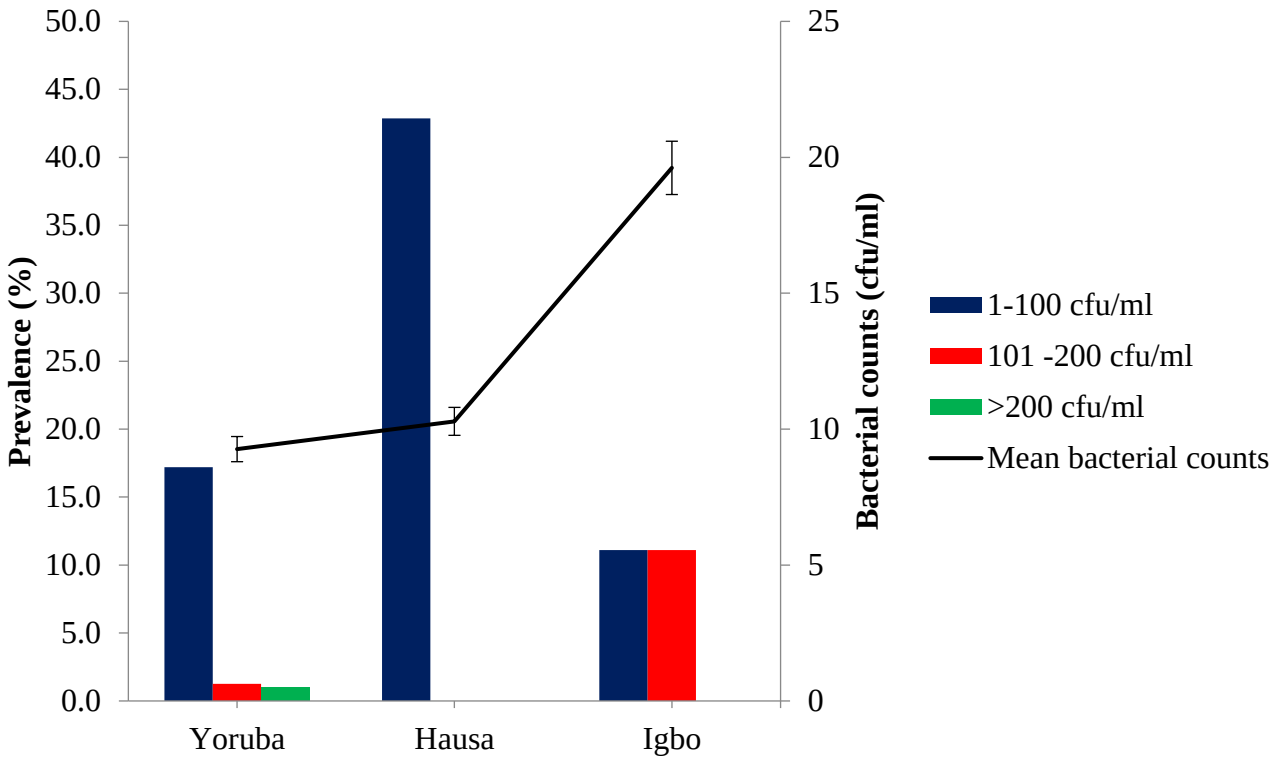

Figure 7: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Tribe

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. (p = 0.029, df= 6, χ^2 = 14.080)

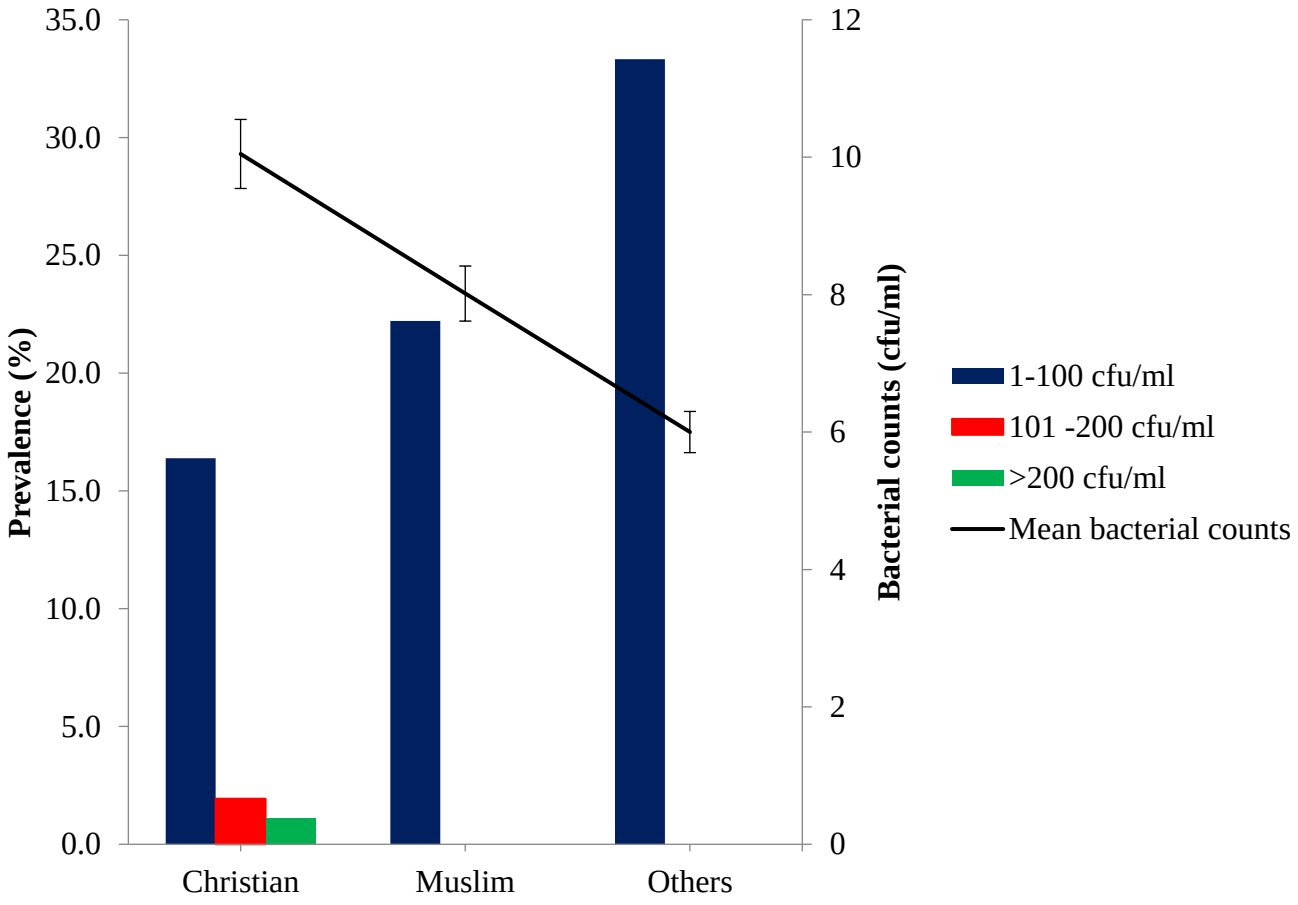

Figure 8: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Religion

Data is presented as percentage prevalence. *p* for trends is significant at 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.

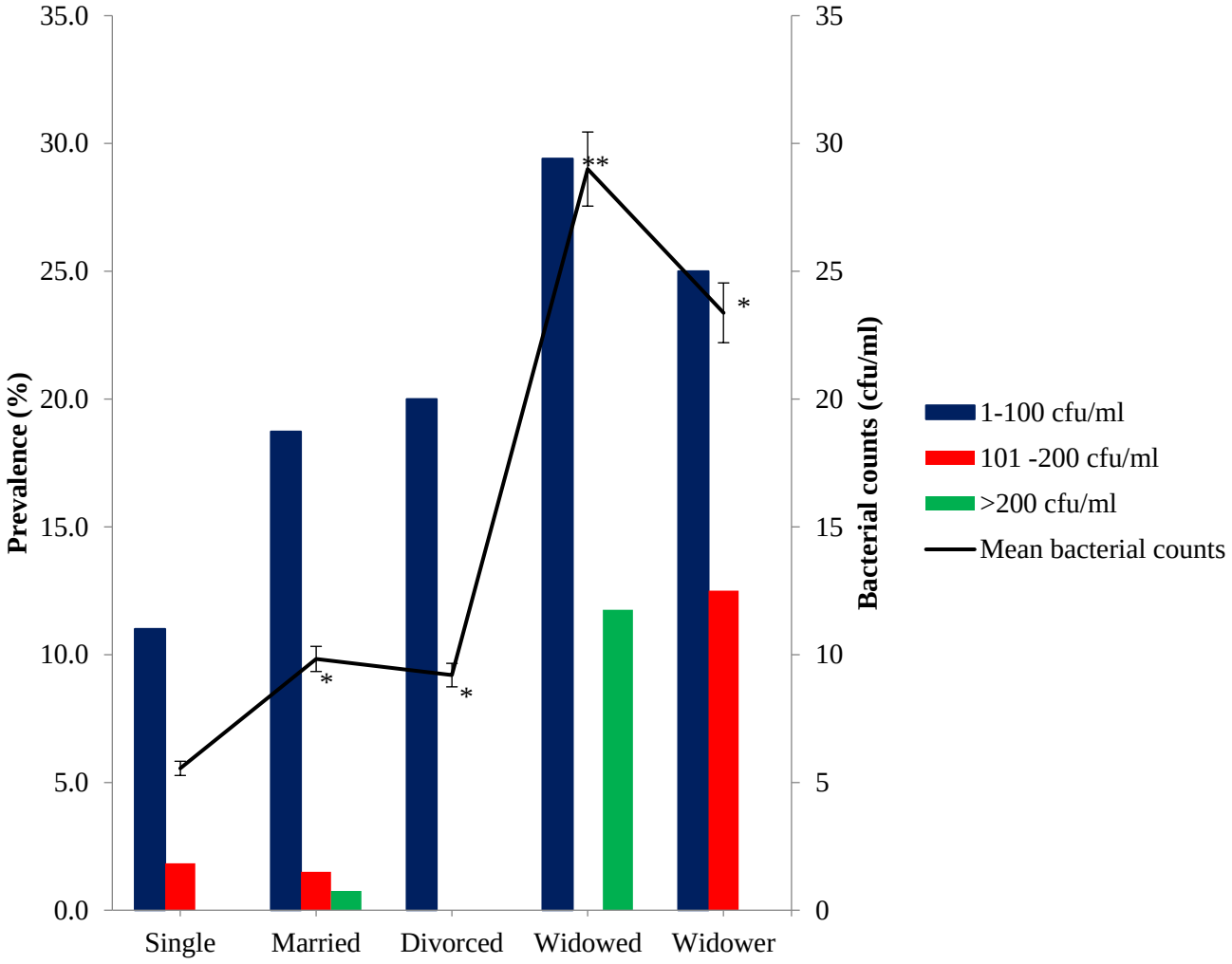

Figure 9: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State in Relation to Marital Status

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. ($p = <0.001$, $df = 12$, $\chi^2 = 35.737$)

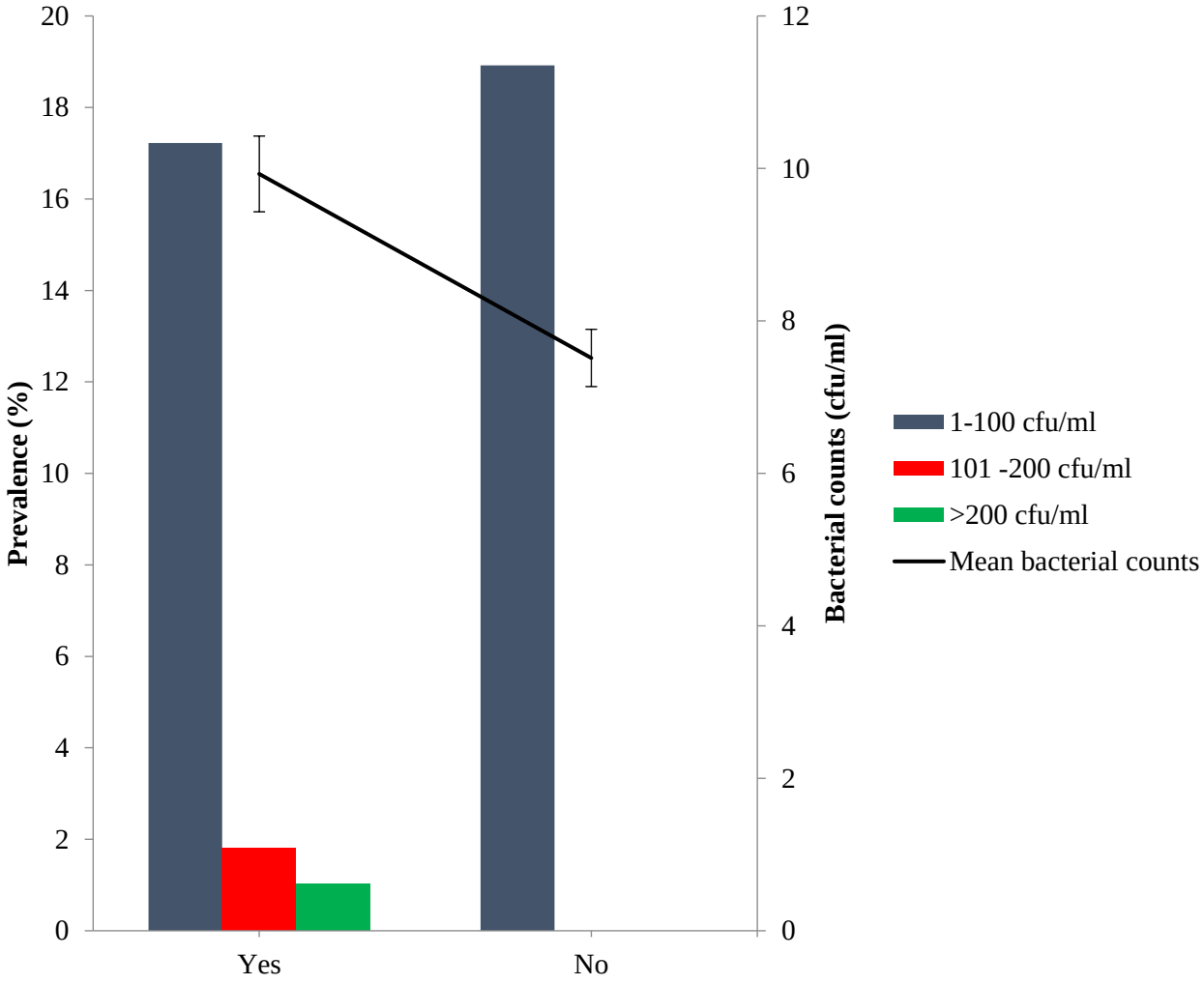

Figure 10: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of Commercial antimalarial drug

Data is presented as percentage prevalence. *p* for trends is significant at 0.05. (p = 0.775, df= 3, χ^2 = 1.108)

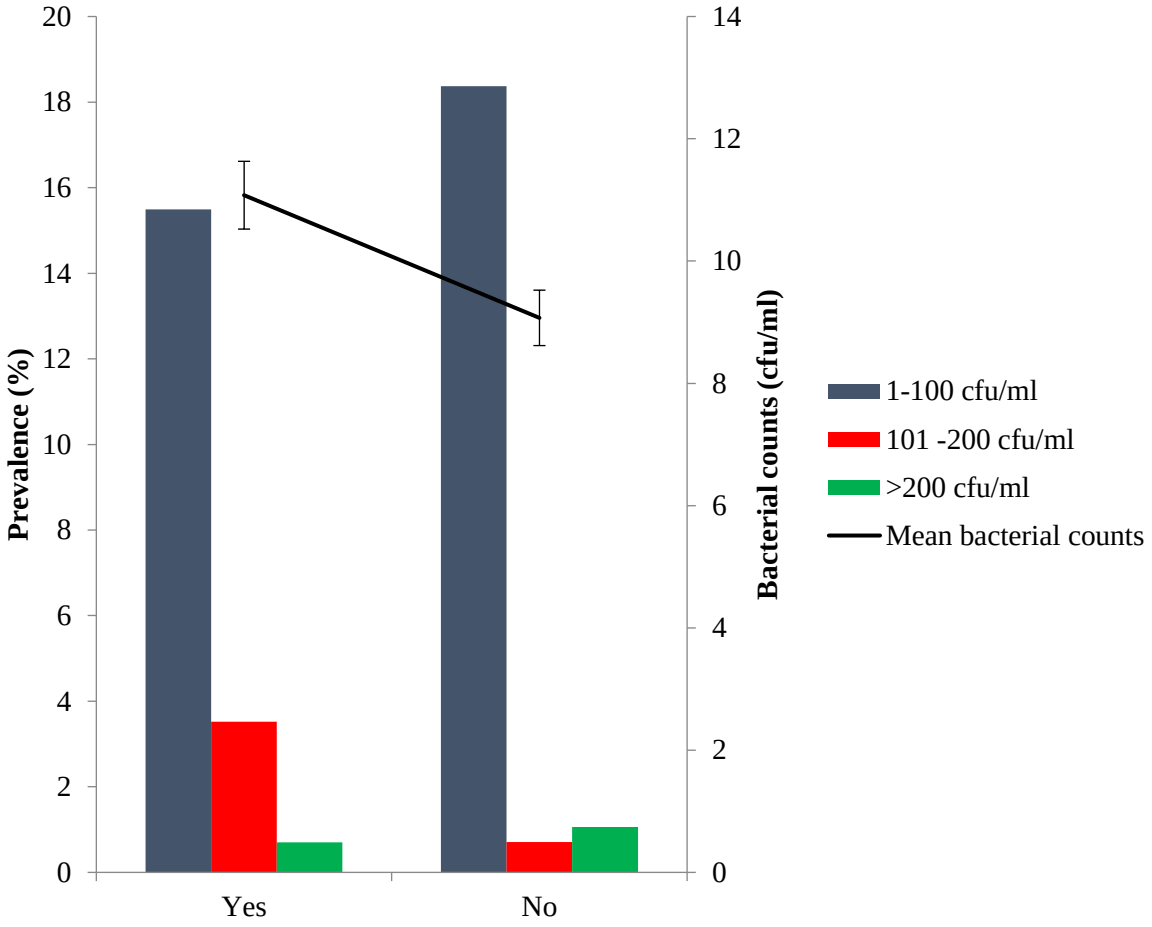

Figure 11: Prevalence of Concomitant Bacteria among Febrile Malaria Patients attending Government Hospital in Ondo State based on the use of Herbs

Data is presented as percentage prevalence. *p* for trends is significant at 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

Concomitant bacterial infection in malaria patients is essential for clinical management and can also contribute to an increased understanding of 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 (Omoya and Ajayi, 2020). Previous studies in Nigeria reported that there was no association between malaria and concomitant bacteria (Christopher and Onaiwu, 2014; Oluyege *et al.,* 2017) and Kenya (Were *et al.,* 2011). 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.

There is strong evidence that recent or current infection with malaria increases the risk of systemic bacterial infections with high associated mortality rates in several sub-saharan countries (Oluyege *et al.,* 2017). The presence of one microorganism generates niche for other pathogenic microorganisms to colonise, in *P. falciparum* infection, alterations in the function of the cells of innate immune system (macrophages, neutrophils, dendritic cells), changes in the adaptive immune system (alteration of B cell population) and regulatory cytokines released during malaria infection to control pro-inflamatory response could impair the mucosal immune response to invasive bacteria leading to blood stream infection (Gomez-Perez *et al.,* 2014).

The prevalence of malaria coinfection found in this study (20.19%), was lower than what was reported in Venezuela (34.2%) (Forero-Peña *et al.,* 2021), India (60%) (Mandage *et al.,* 2020) and corroborate the study reported in Brazil (20%) (Magalhães *et al.,* 2014). 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%) (Birhanie *et al.,* 2014). 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. Thus, physicians should be suspicious of coinfection in malaria cases with inadequate treatment response or atypical manifestations.

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 (Maharath and Ahmed, 2021), it is important to conduct studies to investigate the pathogen loads in this study. In

addition to this, published data for the bacterial load in bloodstream infection in the country is limited and there is a need for baseline studies in this area.

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.* (2014) in Ethiopia and Oluyege *et al.* (2017) in Ekiti, 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 (Alhassan *et al.*, 2012)**.** 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 (Oluyege *et al.,* 2017), 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. Complications were more likely in coinfected patients compared to patients without coinfections, suggesting that coinfection with another pathogen could exacerbate the clinical course of malaria (Forero-Peña *et al*., 2021).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. Also, the prevalence of concomitant bacteria and bacterial load were higher among those that have not taken herbs than those that have taken herbs before visiting the hospital. Though the type of antimalarial drug used by the patients were not considered in this study, however there should be more awareness to discourage the use of non-recommended drug for the treatment of malaria (Simon-Oke *et al.,* 2018) and since antimalarial drug cannot inhibit the growth of bacteria this could have led to the increase in bacteria load. Also, the use of herbs has been known to cure bacterial infection, the higher prevalence observed in this study could be due to differences in the herbs used, and it has been reported that some herbs only suppress microorganisms, lack scientific validation for the treatment and there have not been a well-established recommended dosage for herbal therapy (Ene *et al.*, 2010; Lagnika *et al.*, 2016). Also, Oladunmoye and Kehinde (2011) stated that time of harvest and mode of processing also affect the efficacy of herbs which might have contributed to the high prevalence among the herb user in this study. 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. This study has some limitation in that all the enrolled individuals were febrile patients, then studies enrolling asymptomatic individuals should be performed in the future to evaluate the real burden of coinfections in malaria. Another limitation was the virulence gene of the bacteria were not examined as the ability of some bacteria to invade and survive in the blood could be due to the presence of some virulence genes and not malaria.

Conclusion

In conclusion, this study provides a baseline insight into the prevalence of bacterial concomitant with malaria among the febrile populations in Ondo State. The prevalence of bacteremiea concomitant with malaria was affirmed in this study, the results shows that there is a strong association between malaria and bacteremiea and that malaria appear to predispose febrile patients children to bacteremia in the study area. Level of education, tribe and marital status were significantly associated with bacterial concomitant with malaria. Further studies should be done on the other potential risk factors of malaria and bacterial concomitant in different study areas.

Recommendations

Improved diagnostic, therapeutic, and preventive approaches are urgently needed for bacteremiea concomitant. Antimicrobial resistant patterns of these bacteria has to be presented to aid effective treatment. Delay in either diagnosis or start of therapy for this infection could have fatal outcomes hence self-medication with the use of drugs or herbal products should be discouraged in the study area.

Competing interests

Authors have declared that no competing interests exist.

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