



# Predictors of Comorbidity of Malaria and Septicemia in Children Living in Malaria-Endemic Communities in Nigeria

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## Abstract

**Purpose** The study attempted to identify possible overlap between serum cell-reactive proteins (C-rp) and hematological indices as predictors of comorbidity of malaria and septicemia among children attending primary healthcare facilities in Ilorin, Nigeria.

**Methods** One hundred and ninety-three children (aged:  $\leq 15$  years) presenting with symptoms suggestive of malaria were enrolled. Blood specimens were collected and screened for: Romanowsky, culture, serum C-RP and hematological indices.

**Results** One hundred and fifteen (59.6%) children had *Plasmodium falciparum* infections (female 69.0% and male 34.1%). Septicemia was common among 52 (26.9%), but malaria and septicemia co-infection was 42 (36.5%). C-rp levels were low ( $< 10$  mg/L) in 41 (35.7%, OR 4.594, CI 2.463–8.571) and high ( $> 10$  mg/L) in 74 (64.3%, OR 2.519, CI 1.681–3.775) among the malaria positives ( $p < 0.05$ ). Children with low C-rp, 8 (15.4%, OR 9.413, CI 4.116–21.531) were positive for septicemia and high C-RP 44 (84.6%, OR 1.694, CI 1.396–2.055), but without malaria, respectively. Similarly, increased C-rp levels were significantly associated with clinical malaria;  $> 10,000$  parasites/ $\mu$ L (OR 1.486, CI 1.076–2.054,  $P < 0.001$ ). Malaria-positive versus negative showed that PCV, C-rp, hemoglobin, platelet, WBC, and neutrophil were statistically significant ( $P < 0.05$ ). Two bacteria species were identified, viz; *Staphylococcus aureus* 39 (54.9%) and *Escherichia coli* 32 (45.1%). The trade-off between sensitivity and specificity occurred at 16.475 cut-off using C-rp and degree of malaria severity as the standard for AUROC.

**Conclusion** C-rp are inflammatory markers, though non-specificity may be associated with malaria prognosis and severity during malaria–septicemia co-infection.

**Keywords** Malaria · Septicemia · Cell-reactive proteins · Hematological indices · Nigeria

## Introduction

Malaria remains a life-threatening tropical disease with high morbidity and mortality, especially in children under 5. In time past, several clinical studies identified a plethora of biochemical molecules as biomarkers in malaria-infected individuals with distinct outcomes and manifestations associated with these biomarkers [1]. Knowledge on a number of these biomarkers play significant roles in the routine patient care in clinical settings [2], as they are often used as guide for the

clinician to determine specific and reliable clinical decision-making, and remains vital in epidemiological studies [3]. Cell-reactive protein (C-rp) is an acute-phase reactant in which the hepatocyte synthesis is characterized and modulated by interleukins and tumor necrosis factor (TNF- $\alpha$ ) [4]. It is elevated following many infections involving acute tissue damage and disorders, especially during invasive bacterial infections [5]. Clinically, C-rp is often used as a biomarker for inflammation and functionally, a key component of the innate immune response [6]. It mediates phagocytosis by activating the complement cascade and cytokines, respectively [5]. It is a non-specific reactant but forms complexes by binding to a range of molecules, such as precipitating somatic C polysaccharides and pepto-polysaccharides on bacteria, parasites, and fungi [5]. Symptoms of malaria are unspecific and share common symptoms with several other infectious diseases with symptoms such as chills and rigor,

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loss of appetite, malaise, pallor, hyperthermia, and fever [7]. Therefore, the presence of viral and/or bacterial infections might be misleading as the aforementioned often climax into high serum C-rp titer [8]. Thrombocytopenia occur in up to 80% malaria patients and correlates with severe malaria because platelets kill infected rbc (iRBCs) and activates the protective immune response [9]. On the other hand, the absence of platelets contributes to pathogenesis [10]. During malaria infections, host response against the *Plasmodium* parasite builds up a balance of inflammation, tissue damage, and immune-mediated events that are targeted toward controlling further parasite growth [9]. Hematological abnormalities consistently accompany malaria infections, with the patient presenting with anemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis, and rarely disseminated intravascular coagulation [11]. Binding platelets to the RBCs releases an anti-microbial protein referred to as platelet factor 4 (PF4), this protein accumulates in the RBCs and subsequently lysis the food vacuole of the parasite [9]. Most often in clinical examinations, platelet count is inversely related to the degree of parasitemia [12]. The total WBC count in malaria-infected patients have been more or less inconsistent, because mild leukocytosis and leucopenia occur differently in different clinical settings. Leukocytosis occurs more often during severe *falciparum* malaria and it is associated with poor prognosis [13]. Neutrophils (polymorphonuclear cells) are the first lines of immune defense mediators during malaria and bacterial infections [14, 15]. They phagocytize *Plasmodium* parasites by producing reactive oxygen species (ROS) and other anti-microbial agents [15]. However, some bacterial pathogens have a means of evading death by neutrophils, they do this by producing virulence molecules [16–18]. The study was carried out to establish the relationship, interaction, and possible overlap between elevated titer of serum C-reactive protein and hematological pathogenesis as predictors of comorbidity of malaria and septicemia among children attending primary healthcare facilities in Ilorin, Nigeria.

## Study Design

A hospital-based randomized sampling of febrile children aged  $\leq 1$ –15 yrs were enrolled at the children specialist hospital, Centre-Igboro, Ilorin, Nigeria. Data were collected with pretested questionnaire, patients with history of fever, normal temperature ( $\geq 36.5$ – $37.5$  °C) and or abnormal temperature referred to as hyperthermia ( $> 37.5$  °C) or hypothermia ( $\leq 36$  °C) during the 24 h preceding the consultation were included in the study. The acute and/or severe malaria diagnosis was assessed through parasitological diagnosis using microscopy and or rapid diagnostic tests evaluation and children fulfilling the following inclusion criteria

were enrolled: (i) fever, (ii) positive microscopic examination. Children presenting with other manifestations besides malaria were excluded from the study. Malaria parasitemia was graded as low parasitemia ( $\geq 1$  to  $< 10,000$  parasites/ $\mu\text{L}$ ), moderate parasitemia ( $\geq 10,000$  to  $< 100,000$  parasites/ $\mu\text{L}$ ), and severe parasitemia ( $\geq 100,000$  parasites/ $\mu\text{L}$ ) according to WHO criteria [19].

## Blood Sampling

A Phlebotomist was assigned to collect peripheral and venous blood samples into vacutainer tubes (EDTA anti-coagulant bottles) and plain screw cap bottles.

## Parasitological Examination by Microscopy

*Plasmodium* species and counts (number of parasites per 200 leukocytes) were analyzed by light microscopy and recorded after the methods of Cheesbrough [20]. Briefly, thick blood films were made on clean microscope slides and allowed to air-dry. Each slide was stained with 10% Giemsa solution for 45 min, rinsed with clean water and air-dried. They were then examined under oil immersion microscope. A blood film is regarded as positive if it indicates the presence of asexual blood stage of *Plasmodium* species. Any film with no parasite after examining at least 200 fields was considered negative. *Plasmodium falciparum* was identified in the prepared slides by the presence of Maurer's clefts (membrane limited vacuoles or sack-like structures in the cytosol) and visible blue dots in Giemsa-stained blood smears) and the presence of a high-grade parasitemia in infected red blood cells (iRBCs) and rarely crescent-shaped gametocytes. All slides were double-checked by different certified microscopist and an average of counts was calculated to ensure accuracy.

## Semi-quantitative Determination of Cell-Reactive Protein

About 0.5 mL of venous blood was collected in plain vacutainer bottles and analyzed for quantitative-based immunoassay for the determination of C-rp in whole blood, serum or plasma. The test was standardized to detect C-rp levels below or above 10 mg/L, as described by Sarfo *et al.* [21]. Briefly, about 10 and 12 drops of the buffer solution included in the test kit was carefully put in a test tube, by holding it up vertically to prevent air trapping. A capillary tube was filled with approximately 3.5  $\mu\text{L}$  of the patient's serum and subsequently transferred into the test tube initially filled with buffer solution. The mixture was vortexed thoroughly. Three drops of the specimen mixture were then placed in the specimen well, and after about 5 min, the results obtained by the appearance of the content were interpreted. The appearance

of a line in the control marked “C” confirmed the accuracy of the test. In the middle part of the test is the reference line “R”, with the test line “T” closest to the test field “S”. The results were then interpreted based on the colored lines which appeared in the field lines. A test was said to be positive when three distinct colors appeared in the lines “T”, “C”, and “R”. The C-rp concentration of a sample is interpreted as 10–30 mg/L when the test line (T) is fainter than the reference line (R), around 30 mg/L when the test (T) is close to the reference line (R) in intensity and over 30 mg/L when the test line (T) is brighter than the reference line (R). The C-rp concentration in the sample was less than 10 mg/L and the test was considered negative when two colored lines appeared in the control (C) and reference (R) areas, with no distinct color line appearing in the test area (T). A test was considered invalid when there was no control line (C) and/or reference line (R). C-rp levels with titres of < 10 mg/L were considered low and those with > 10 mg/L were considered high.

### Estimation of Packed Cell Volume (PCV)

Packed cell volume (PCV) of the volunteers was examined out according to the methods described by Dacie and Lewis [22] with slight modifications. Briefly, approximately 2 mL of blood samples were collected from the patients into EDTA vials and subsequently subjected to centrifugal force to allow the cells become packed in the distal end of the vials. The centrifuged blood was then re-introduced into microhematocrit (capillary) tube up unto about 75% of its length, and sealed with clay sealant at both ends of the tube (commonly 75 mm long, 1 mm diameter). The blood-filled microhematocrit tubes were introduced into a microhematocrit centrifuge at 10,000 revolutions per minute (rpm) and relative centrifugal force (rcf) at 15,000 g for 5 min, to separate the blood into layers. A microhematocrit reader was used to read the meniscus of the RBC as the PCV in percentage. Anemia was defined as PCV < 30%, mild anemia PCV 25–29%, moderate anemia PCV 19–24%, severe anemia PCV < 18%, and control anemia PCV  $\approx$  40% (control group).

### Determination of Septicemia

#### Blood culture

Specimens of blood obtained from venipuncture contained in plain bottles were cultured separately. The samples were introduced into broth medium and analyzed at the University of Ilorin Veterinary Teaching Hospital, Ilorin. Briefly, on day 1, the samples were collected into a sterile sample bottle containing a non-selective growth medium (Buffered peptone water), mixed gently and incubated at 37 °C for

24 h. On day 2, 10 mL loop full from the inoculated and incubated buffered peptone water were spread and streaked aseptically on noon selective enrichment and basal medium (blood agar, nutrient agar), plates were incubated at 37 °C overnight (18–24 h); on day 3, the colonial characteristics of the suspected bacteria growth and sub-cultivation were read to observe the different morphology of the bacterial growth. The observable Gram-positive and Gram-negative bacterial colonies (i.e., with a zone of clear (beta-type), hemolysis produced by alpha toxin) were inoculated on various selective and different agar plates (MacConkey agar, mannitol salt agar, and eosin methylene blue agar), incubated at 37 °C overnight (18–24 h). On day 4, all the aforementioned plates were read for morphological characteristics. The biochemical evaluation of the bacterial species was also carried out using the coagulase test specifically to confirm the presence of pathogenic *Staphylococcus aureus* in the broth medium. Briefly, the broth medium was mixed into a drop of EDTA-treated rabbit plasma on a microscope slide and the result showed a degree of coagulation, from a loose clot of bacteria colony suspended in plasma, forming a solid immovable clot considered to be a positive result. The agar plates were dried in the oven to remove moisture contents. The culture technique was carried out by streak method. The plates were streaked aseptically with sterile wire loops to form discrete colonies after the methods of Cheesbrough [20]. Further identification of pathogens was carried out following Christopher *et al.* [23] and Mshana *et al.* [24].

### Determination of Hematological Parameters

A complete blood count (CBC) involving the following constituents was determined with an auto-hematology analyzer (Sysmex KX-21N), viz; hemoglobin, packed cell volume (PCV), red blood cell (rbc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), platelet, neutrophil, and lymphocytes. The CBC procedures were carried out after the methods of Bhandari *et al.* [25]. Briefly, approximately 1 mL of blood sample were collected aseptically from each subject into tri-potassium ethylene-diamine tetra-acetic acid (K<sub>3</sub>EDTA) anti-coagulant bottle. The anti-coagulant bottle was inverted and mixed gently to ensure complete blood count (CBC) analysis.

#### Data Analysis

The statistical analysis was carried out with IBM SPSS 20.0 Statistics and GraphPad Prism 7 version 7.04. All categorical data were presented as frequency (percentages), and the Chi-square test statistics was used to test for association between variables. The Mantel–Haenszel test of trend was used to examine rates across levels of parasitemia. Continuous

variables were presented as mean  $\pm$  standard deviation for parametric variables and median (interquartile range) for nonparametric variables. The Kruskal–Wallis test was used to assess significance of differences for nonparametric variables between control (PCV) and the different grades of malaria parasitemia, followed by the Bonferroni post hoc multiple comparison test. Partial correlation was used to test for associations between various parameters upon adjusting for age and gender. The ROC analysis was used to ascertain the diagnostic performance of C-rp for malaria. Confidence was set at 95%, and the  $P$  value of less than 0.05 was considered statistically significant. Spearman's test was used to examine the correlation between parasite density and platelet counts. Proportions were compared by the Fisher's exact test. Thrombocytopenia was defined as a platelet count of less than 150,000/ $\mu$ L.

## Results

One hundred ninety-three (193) febrile children aged  $\leq 1$ –15 yrs were randomly selected and enrolled for this study. The distribution of the study enrollment with respect to gender was as follows: males 65 (33.7%) and females 128 (66.3%) (Table 1).

All malaria-positives had mono infections of *Plasmodium falciparum* with a prevalence rate of 59.6%. Children aged  $< 1$ –5 yrs had the following prevalence with respect to gender (female 69.0% and male 34.1%) while children aged 6–15 yrs recorded the following prevalence with respect to gender (female 31.0% and male 65.9%). However, the association was not statistically significant with respect to gender ( $P=0.102$ ) but was significant with respect to age groups ( $P=0.004$ ) (Fig. 1).

Fifty two (52) children were infected with septicemia among the total population (193) examined. Out of the malaria-positive children (115), 42 (36.5%) were infected with both malaria and septicemia. Among the age group  $\leq 1$ –5 yrs that were females, 57.9% had both infections and among the male with age groups 6–15 yrs, 60.9% carried both infections (Fig. 2).

The univariate analysis of the association between C-rp levels, malaria prevalence, and septicemia among the study participants shows the relevance and association between the groups. C-RP levels were low ( $< 10$  mg/L) in 41 (35.7%, OR 4.594, CI 2.463–8.571) and high ( $> 10$  mg/L) in 74 (64.3%, OR 2.519, CI 1.681–3.775) among the malaria-positives with a statistically significant association ( $P=0.001$ ). However, out of the 16 individuals with low C-rp, 8 (15.4%, OR 9.413, CI 4.116–21.531) were positive for septicemia, and high C-rp levels were observed in 44 (84.6%, OR 1.694, CI 1.396–2.055) among this group. Similarly, increased C-rp levels were strongly associated with (OR 1.486, CI

**Table 1** Basic characteristics of the study enrolments ( $n=193$ )

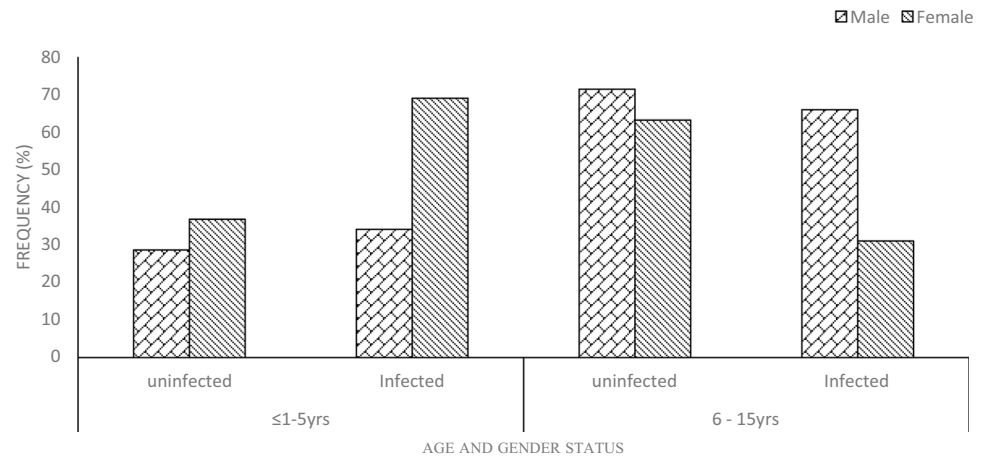
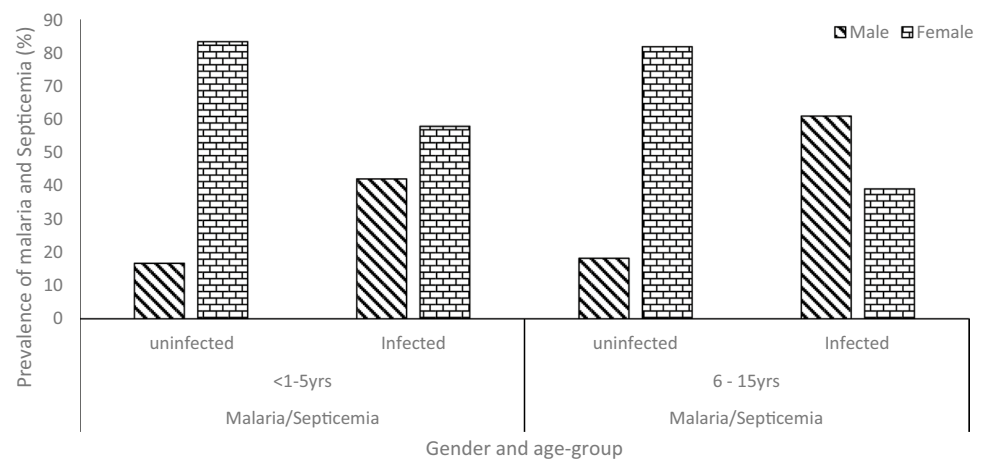
| Characteristics               | Sex $n$ (%) |            | $P$ value |
|-------------------------------|-------------|------------|-----------|
|                               | Male        | Female     |           |
| Age group (yrs.)              |             |            | $< 0.01$  |
| $\leq 1$ –5                   | 21 (23.1)   | 70 (76.9)  |           |
| 6–15                          | 44 (43.1)   | 58 (56.9)  |           |
| Total                         | 65 (33.7)   | 128 (66.3) |           |
| Axillary temperature          |             |            | N.S       |
| Normal <sup>a</sup>           | 9 (13.8)    | 19 (14.8)  |           |
| Hypothermia <sup>b</sup>      | 46 (70.8)   | 89 (69.5)  |           |
| Hyperthermia <sup>c</sup>     | 10 (15.4)   | 20 (15.6)  |           |
| Hematocrit                    |             |            | $< 0.001$ |
| Control (40%)                 | 26 (40.0)   | 89 (69.5)  |           |
| Mild (25–29%)                 | 39 (60.0)   | 32 (25.0)  |           |
| Moderate (19–24%)             | 0           | 4 (3.1)    |           |
| Severe ( $< 18\%$ )           | 0           | 3 (2.3)    |           |
| C-reactive protein (mg/L)     |             |            | $< 0.001$ |
| $< 10$                        | 8 (12.3)    | 89 (69.5)  |           |
| $> 10$                        | 57 (52.5)   | 39 (30.5)  |           |
| Hemoglobin (g/dL)             |             |            | N.S       |
| Normal (10.6–15.5)            | 56 (86.2)   | 107 (83.6) |           |
| Low ( $< 10.5$ )              | 9 (13.8)    | 21 (16.4)  |           |
| Polycythemia ( $> 15.6$ )     | 0           | 0          |           |
| Platelets ( $10^9/\mu$ L)     |             |            | N.S       |
| Normal <sup>d</sup>           | 50 (76.9)   | 111 (86.7) |           |
| Thrombocytopenia <sup>e</sup> | 15 (23.1)   | 17 (13.3)  |           |
| Thrombocytosis <sup>f</sup>   | 0           | 0          |           |
| Malaria status                |             |            | N.S       |
| Infected                      | 44 (67.7)   | 71 (55.5)  |           |
| Not infected                  | 21 (32.3)   | 57 (44.5)  |           |
| Septicemia                    |             |            | $< 0.001$ |
| Infected                      | 31 (47.7)   | 21 (16.4)  |           |
| Not infected                  | 34 (52.3)   | 107 (83.6) |           |

<sup>a</sup> $> 36.5$ – $37.5$  °C; <sup>b</sup> $\leq 36$  °C; <sup>c</sup> $> 37.5$ ; <sup>d</sup>150,000–450,000; <sup>e</sup> $< 150,000$ ; <sup>f</sup> $> 450,000$

N.S—not significant because the  $P$  value is above the 0.05 threshold

1.076–2.054,  $P < 0.001$ ). Blood culture results were available for 193 individuals, and out of this population, 52 (36.9%) patients were positive for *Staphylococcus aureus* and *Escherichia coli* infections with 8 (15.4%) recording  $< 10$  mg/L C-RP levels (Table 2).

The geometric mean of the blood parameters was evaluated between malaria-positive and malaria-negative population in children age  $\leq 1$ –15 yrs. PCV, C-rp, hemoglobin, platelet, WBC, and neutrophil were observed to be statistically significant ( $P < 0.05$ ), but axillary temperature in relation to malaria and septicemia, respectively, was marginally significant ( $P = 0.05$ ) and lymphocyte titre was also not comparable ( $P = 0.44$ ) to both the geometric means of malaria and septicemia. However, septicemia-positive and

**Fig. 1** Prevalence of malaria infection with respect to age and gender**Fig. 2** Co-prevalence of malaria–septicemia co-infection with respect to age and gender**Table 2** Univariate analysis of the association between C-RP levels, malaria prevalence, and septicemia among selected children age: < 1–15 yrs in the study

| C-RP      | Malaria (%) |           |                     | Septicemia (%) |           |                      | Parasitemia n (%) |           |                     |
|-----------|-------------|-----------|---------------------|----------------|-----------|----------------------|-------------------|-----------|---------------------|
|           | Uninfected  | Infected  | OR (95% CI)         | Uninfected     | Infected  | OR (95% CI)          | UCM               | CM        | OR (95% CI)         |
| < 10 mg/L | 56 (71.8)   | 41 (35.7) | 4.594 (2.463–8.571) | 89 (63.1)      | 8 (15.4)  | 9.413 (4.116–21.531) | 28 (45.2)         | 13 (24.5) | 2.534 (1.137–5.645) |
| > 10 mg/L | 22 (28.2)   | 74 (64.3) | 2.519 (1.681–3.775) | 52 (36.9)      | 44 (84.6) | 1.694 (1.396–2.055)  | 34 (54.8)         | 40 (75.5) | 1.486 (1.076–2.054) |
| Total     | 78          | 115       | 0.548 (0.424–0.709) | 141            | 52        | 0.180 (0.089–0.362)  | 62                | 53        | 0.587 (0.357–0.963) |
| P value   | 0.001       |           |                     | 0.001          |           |                      | 0.001             |           |                     |

C-rp: cell-reactive protein; < 10 mg/L: low C-RP; > 10 mg/L: high C-RP; UCM: uncomplicated malaria (1–99,999); CM: complicated malaria (> 100,000) asexual parasites [19]

non-septicemic populations were not comparable except in C-rp, WBC, and neutrophils ( $P < 0.05$ ) (Table 3).

Malaria severity among the malaria-positives was further stratified with reference to the blood profiles and this showed individuals with low (25); moderate (39), and severe (51) infections. C-rp (mg/L) levels among the individuals with light infections had a mean  $\pm$  SE. error, median, variance,

range, and IQR of  $9.187 \pm 1.648$ , 5.05, 67.91, 28.04, and 13.392, respectively, while those with moderate infections had  $17.494 \pm 1.630$ , 21.314, 103.56, 31.25, and 20.083 and severe infections had  $17.558 \pm 1.490$ , 17.775, 113.27, 35.78, and 19.820, respectively. The platelet count ( $10^9/L$ ) also had the following statistics; low ( $265.40 \pm 22.116$ , 276.00, 12,230.00, 541.00, 134.00), moderate ( $276.87 \pm 16.825$ ,

**Table 3** Geometric mean of blood parameters with respect to malaria and septicemia status among the study enrolments

| Parameters | Malaria          |               |                     |               | Septicemia      |                |                      |         |
|------------|------------------|---------------|---------------------|---------------|-----------------|----------------|----------------------|---------|
|            | Infected (N=115) |               | Non-infected (N=78) |               | Infected (N=52) |                | Non-infected (N=141) |         |
|            | Mean ± St. dev   | 95% CI        | Mean ± St. dev      | 95% CI        | P value         | Mean ± St. dev | 95% CI               | P value |
| Temp       | 36.83 ± 1.35     | 36.58–37.08   | 37.13 ± 0.29        | 37.07–37.20   | 0.05            | 36.95 ± 0.86   | 36.71–37.19          | 0.96    |
| PCV        | 34.63 ± 4.82     | 33.74–35.52   | 36.10 ± 2.49        | 35.54–36.66   | <0.01           | 36.45 ± 2.93   | 35.63–37.26          | 0.11    |
| C-rp       | 15.72 ± 10.51    | 13.78–17.66   | 8.93 ± 9.20         | 6.86–11.01    | <0.001          | 1.658 ± 7.98   | 14.36–18.80          | <0.01   |
| Haem       | 10.99 ± 1.74     | 10.67–11.31   | 11.60 ± 0.80        | 11.42–11.78   | 0.01            | 11.43 ± 1.46   | 11.03–11.84          | 0.25    |
| Plat       | 234.60 ± 116.69  | 213.05–256.16 | 176.48 ± 12.16      | 173.74–179.22 | <0.001          | 199.9 ± 101.6  | 170.9–227.4          | 0.29    |
| WBC        | 9.42 ± 4.93      | 8.51–10.33    | 3.59 ± 0.25         | 3.53–3.64     | <0.001          | 8.615 ± 5.217  | 7.163–10.06          | <0.01   |
| Neu        | 47.32 ± 9.26     | 45.61–49.03   | 43.74 ± 3.01        | 43.06–44.42   | <0.01           | 50.17 ± 8.46   | 47.81–52.52          | <0.001  |
| Lym        | 50.05 ± 18.94    | 46.56–53.55   | 52.33 ± 20.56       | 47.69–56.96   | 0.43            | 49.17 ± 20.55  | 43.45–54.8           | 0.44    |

253.00, 11,040.00, 631.00, 120.00), and severe ( $187.180 \pm 15.734$ , 141.00, 12,630.00, 457.00, 151). Differences in axillary temperatures were incomparable (Fig. 3).

The geometric mean of certain blood entities was also compared with respect to their septicemia status (Fig. 4). The following observations were recorded, C-rp (mg/L) levels among the septicemia-positive individuals with mean ± SE, median, variance, range, and IQR of  $17.085 \pm 1.261$ , 17.287, 66.76, 31.25, and 12.289, respectively, while non-infected had  $14.929 \pm 1.361$ , 13.41, 135.13, 36.47, and 21.47. The platelet count ( $10^9/L$ ) also had the following statistics; infected:  $201.40 \pm 17.456$ , 174.00,  $1.280 \times 10^4$ , 394.00, 200.00 and non-infected:  $253.71 \pm 13.477$ , 250.00,  $1.326 \times 10^4$ , 631.00, 138.00.

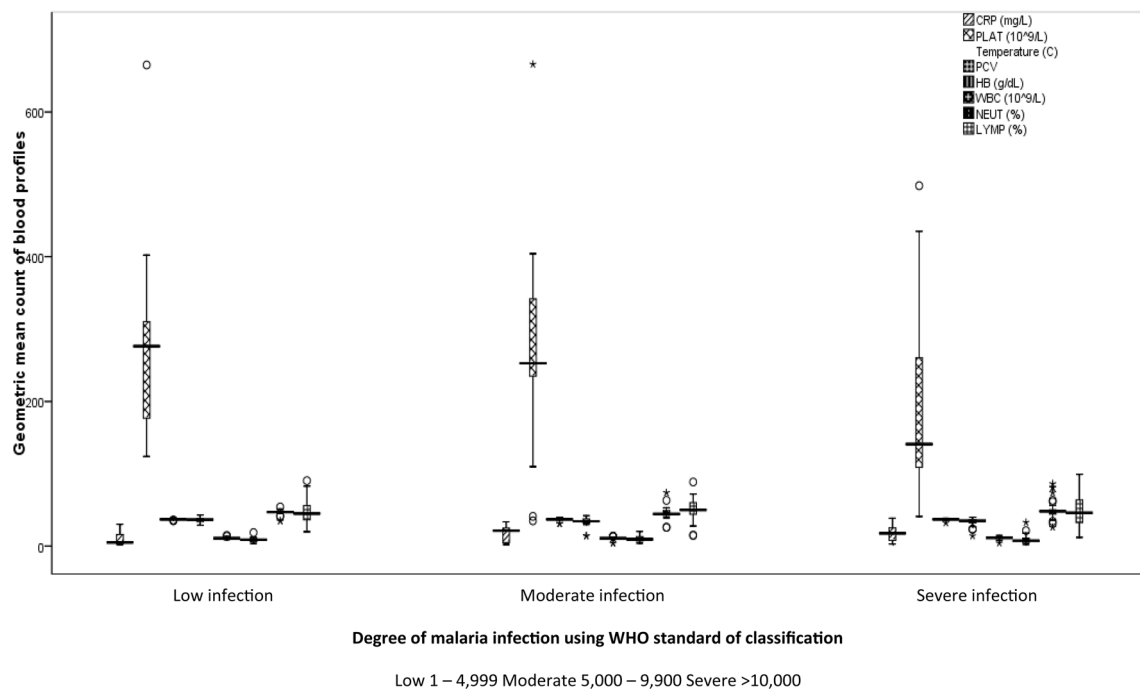
Two bacteria species were identified, viz; *Staphylococcus aureus* 39 (54.9%) and *Escherichia coli* 32 (45.1%), with a co-infection of both bacteria being 19 (16.5%). Of the septicemic individuals, more had co-infection with malaria: *Escherichia coli* 11 (84.6%), *Staphylococcus aureus* 12 (60.0%). The association was, however, statistically significant ( $P < 0.05$ ) (Table 4).

Figure 5 shows some of the isolated bacteria species obtained from blood culture exhibiting various colony forms with precipitated bile salt typical with *E. coli*.

In Fig. 6, C-rp levels for malaria light infection follows the right-hand border indicating a poor fit for the C-rp as observed in that category ( $> 0.05$ ). In C-rp levels for moderate malaria infection, the area under the curve (AUROC) shows an improved detection level of accuracy, whereby the best fit of the highest sensitivity and lowest 1-specificity occurred at 16.475 cut-off. C-rp levels for severe malaria (highest parasitemia) were favorably skewed to the left-hand corner.

## Discussion

The findings stemming from the present study validate Nigeria as one of the malaria hyper-endemic region where children experience frequent, but mild-to-moderate episodes of malaria infections all year round [26, 27]. Hypothermia was observed among the studied population, and this health condition may be due to the inhabitants' habit of self-medication before presentation at the clinic. Antipyretic drugs reduce axillary temperature in malaria-febrile children so as to guide against convulsion and other clinical syndromes [28]. In addition, septicemia-positive patients usually present with hypothermia as an early clinical predictor of sepsis-induced immunosuppression [29]. Previous studies have shown that hypothermic conditions have the potentials to delay the erythrocytic life cycle development of *P. falciparum* [30, 31]. Hyperthermia was observed among some of the febrile children, and to date, febrile temperature

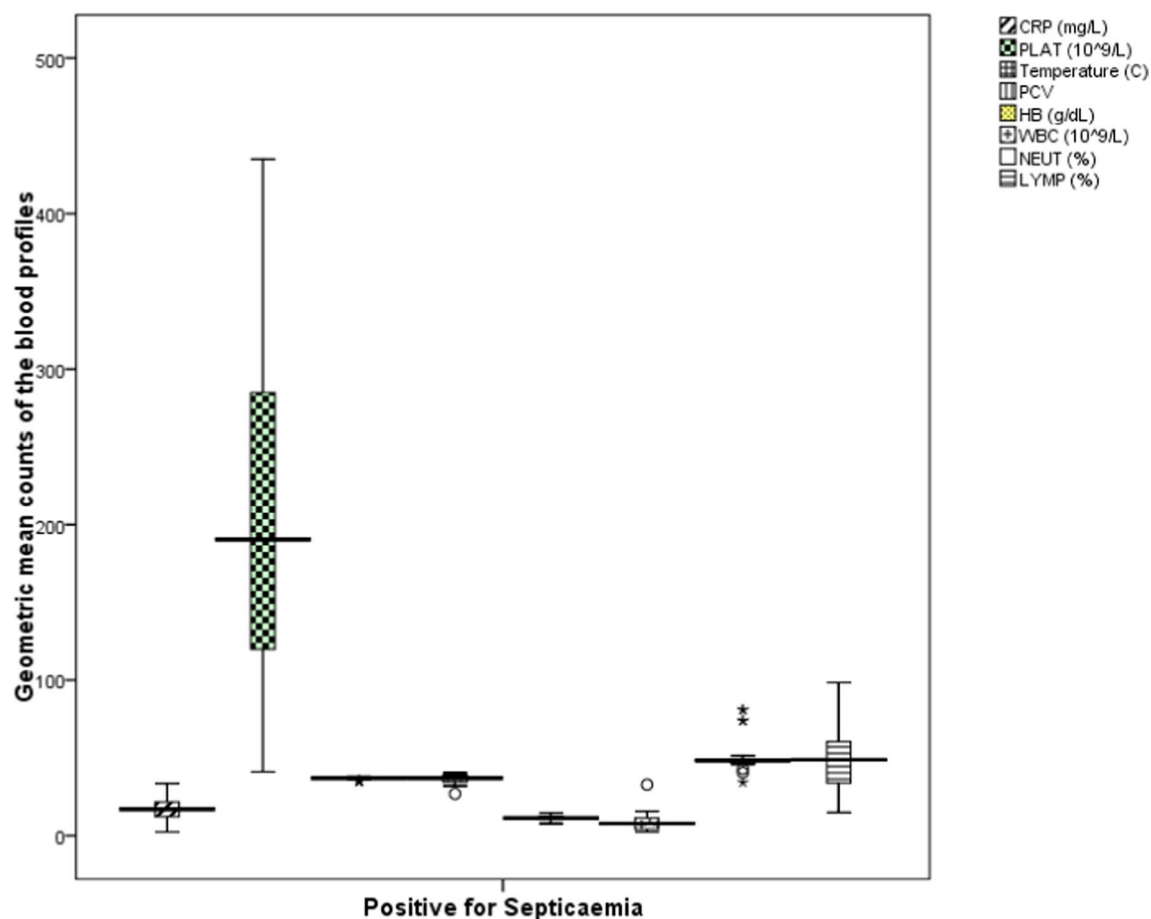


**Fig. 3** Geometric mean counts of blood profiles with respect to the degree of malaria status among the malaria-positives in the study. Boxes represent 25th–75th percentiles, solid bar within boxes represent median values, values within the bars represent non-outlier max-

imum and minimum, mild (black circle), moderate (white circle), and extreme (asterisk) outliers. (Alpha levels of geometric mean of blood profiles for the Pf malaria-positives categories were statistically significant @  $P < 0.05$ )

(> 37.5 °C) is associated with inhibited parasite growth [31]. These observations imply that the appearance of repeated febrile episodes in malaria patients does hasten intra-erythrocytic development of the parasite, thereby justifying the regulatory role played by temperature in *Plasmodium* species intra-erythrocytic cycle [32–34]. On the contrary, previous studies demonstrate that hypothermic conditions (28–32 °C) delayed the erythrocytic life cycle development of *P. falciparum* [35], whereas febrile temperature (40 °C) inhibited parasite growth *in vitro* [36]. Alphard *et al.* [37] reported that hyperthermic patients with malaria had a lower incidence of syphilis; in order words, high fever impedes infectious agents. This probably explains in the current study, why some of the children presenting with axillary temperature above > 37.5 °C had no septicemia. Two major syndromes associated with malaria-related morbidity in children include cerebral malaria and severe malarial anemia. The pattern of both aforementioned syndromes varies and it is dependent on the transmission intensity, for instance, cerebral malaria is most peculiar to children domiciling in areas with low malaria parasite transmission, whereas comorbidity of severe malaria-anemic conditions is common to children above 2 yrs of age domiciling in areas with intense transmission [38]. Although this study did not identify any case of cerebral malaria, only a few cases of mild and severe anemia were associated with peripheral malaria parasite infection,

which is a similar occurrence reported by Babalola *et al.* [39]. Supposedly, malaria may exacerbate anemia through the parasitic destruction of red blood cells or may be as a result of other confounding infections. The disease often induce dyserythropoiesis and reticulocytosis when the infection is not redressed early [40]. From our study, malaria-positive children had reduced hemoglobin concentration which potentiate that hemolysis is a resultant effect of malaria in areas of high malaria transmission [40]. It can also be attributed to the loss of parasitized and unparasitized erythrocytes [41, 42]. Alterations in the hematological constituents of the host are common complications during malaria and it plays major role in the pathogenesis of the disease [12]. The study observed that malaria-positive children had significantly lower PCV, hemoglobin, platelets, WBC, and neutrophil. Our finding is consistent with other studies [12, 43]. However, the decrease in neutrophil count in malaria-positive children as observed in the current research was in contrast to that of an earlier study where the authors suggested that neutrophil elevation might be supposedly due to one or more of the following; (a) activated neutrophil production, (b) release from the bone marrow, and (c) suppressed peripheral removal [44]. On the contrary, septicemia-positive children only show significance in a few hematological abnormalities, viz; WBC and neutrophil, while all others were not comparable. Our observation negates other similar study as



**Fig. 4** Geometric mean counts of blood profiles with respect to septicemia status among the population sampled for the study. Boxes represent 25th–75th percentiles, solid bar within boxes represent

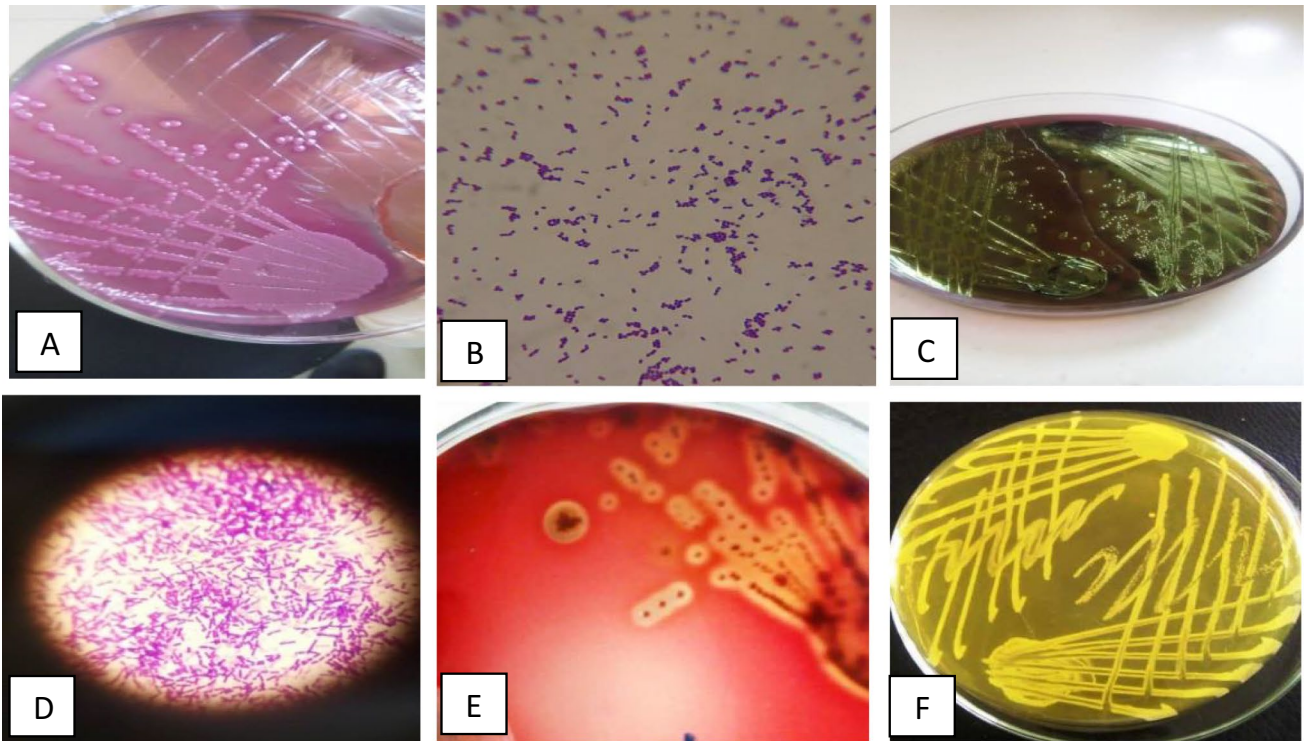
median values, values within the bars represent non-outlier maximum and minimum, mild (black circle), moderate (white circle), and extreme (asterisk) outliers

**Table 4** Malaria prevalence status with respect to blood culture isolates from the study enrollment

| Organism identified                                     | Mal. and pathogens <i>n</i> (%) | Non-mal and pathogens <i>n</i> (%) | <i>P</i> value |
|---------------------------------------------------------|---------------------------------|------------------------------------|----------------|
|                                                         |                                 |                                    | <0.001         |
| <i>Escherichia coli</i>                                 | 11 (84.6)                       | 2 (15.4)                           |                |
| <i>Staphylococcus aureus</i>                            | 12 (60.0)                       | 8 (40.0)                           |                |
| <i>Staphylococcus aureus</i><br><i>Escherichia coli</i> | 19 (100.0)                      | 0                                  |                |
| No septicemia                                           | 73 (51.8)                       | 68 (48.2)                          |                |
| Total                                                   | 115 (59.6)                      | 78 (40.4)                          |                |

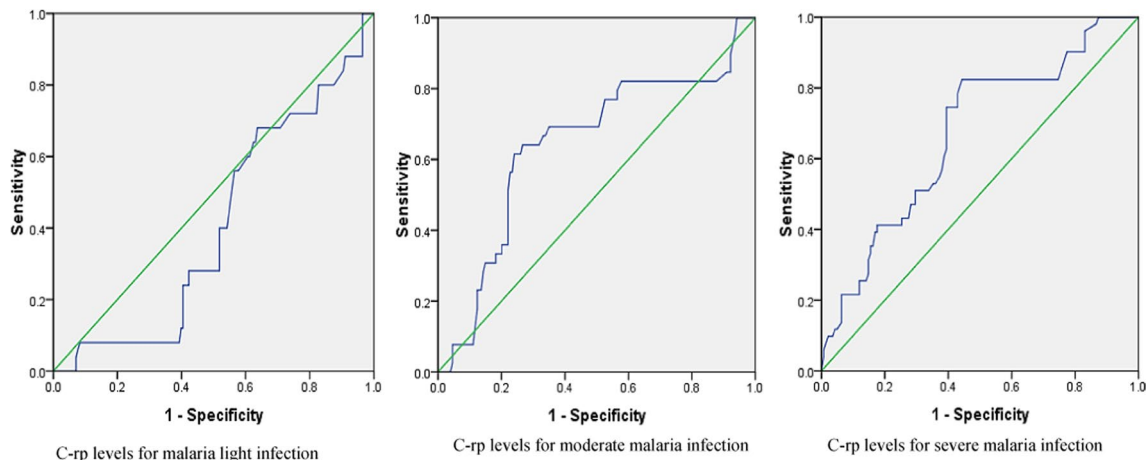
platelet count was a major determinant of thrombocytopenia [45]. However, abnormalities in WBC, differential and platelet counts are not invariably specific for bacterial infection nor do normal values adequately exclude it. At times, blood cultures may be negative even in fatal cases in newborns dying with significant foci of bacterial infections [46]. In

some cases, infection may proceed in the absence of fever, it becomes pertinent to ascertain the onset of hyperthermia as a reliable indication of infection rather than waiting for the manifestation of the underlying disease [47]. We observed from our study, that children with *P. falciparum* malaria were at risk of septicemia. The aforementioned is in conformity with an earlier study where the researchers identified that severe malaria in children is associated with invasive bacterial infection (IBI) [48]. Furthermore, the reviewers asserted that blood stream infections are largely secondary to enteric Gram-negative organisms and were widely reported as a complication of severe malaria. However, it remains uncertain whether malaria infection is a risk factor for invasive bacterial disease since the majority of children in malaria-endemic Africa are frequently infected by *P. falciparum* throughout their childhood years and only a few progress to severe disease [48]. Acute-phase reactant titres of increasing C-rp ( $> 10$  mg/L) were significant in both malaria-positive and septicemia-positive children indicating a possible consequence of inflammation. Increase in blood



**Fig. 5** Pictures of some of the isolated bacteria species identified from septicemia-positive samples. **A** A typical *E. coli* colony on MacConkey agar plates were flat, dried, pinkish in color with a surrounding darker pink of precipitated bile salt. **B** Shape and cellular arrangement of *Staphylococcus aureus* (Cocci in clusters), (Gram-positive cell) 100×oil immersion objective. **C** Typical *E. coli* colonies were small with a greenish metallic sheen appearance on eosin methylene

blue agar plates. **D** Shape and cellular arrangement of *Escherichia coli* (Gram-negative cell). 100×oil immersion objective. Rod arrange singly (**E**)—(a) Cocci in cluster—*S. aureus* on blood agar, (b) rod arranged in singly on blood agar. **F** Typical *Staphylococcus aureus* colonies and the agar plate turned to yellow color due to fermentation of mannitol salt



**Fig. 6** Receiver operating characteristic (ROC) curve of C-rp in relation to the degree of malaria infection among randomized children  $\leq 15$  yrs old in the study

C-rp as an arm of the innate immunity is usually triggered during malaria and bacterial infections [5, 49]. There was an overlap of serum C-rp and hematological abnormalities among the children sampled for this study where both

malaria and septicemia co occurred. C-rp titer was a strong prognostic indicator of malaria severity, but was not the case with septicemia. The best fit of the highest sensitivity and lowest 1-specificity occurred during severe malaria and this

was occasioned by the highest parasitemia count as similar to Sarfo *et al.* [21]. Similarly, in a related study, the usefulness of high-sensitivity C-rp in malaria diagnosis and morbidity in a pediatric population in Ghana was established [8]. Two bacteria species, viz; *Staphylococcus aureus* and *Escherichia coli*, were identified in this study; however, the presence of *E. coli* looks incredible as the pathogen is an enteric parasite but share commonality in other foci [48]. The C-rp cut-off value obtained in this study may serve as a reliable biosensor for malaria morbidity.

## Conclusion

In malaria-endemic regions, miss-diagnosis and presumptive therapeutic practices are injurious to the overall well-being of managing children with malaria-septicemic conditions. Furthermore, establishing the relationship between malaria and septicemia has been a challenging and arduous task to the clinician because contamination remains a possibility in studies reporting incidences of *S. aureus* and *E. coli*. Both infections are common in children domiciling in the tropics presenting with fever in malaria-endemic areas where during the rainy seasons, the victims are predisposed to blood-borne infection alongside enteric pathogens. This study further substantiates evidences suggesting that children with recent or acute malaria may be at risk of bacterial infection with a multiplying effect on common comorbidity. C-rp was established as a common reactant whose plasma concentration increases during malaria-septicemia inflammatory reactions and was found to correlate strongly with *Plasmodium falciparum* parasite densities. Future studies may be pertinent to guide appropriate therapy and provide effective guidelines to make decisions regarding judicious use of antibiotic therapy.

**Author Contributions** OSH carried out the field and clinical analysis, compiled the data, and wrote the first draft. MOO processed the data, performed statistical analyses, and edited the draft manuscript. TAO supervised the entire research and wrote the final draft. All the authors read and edited the paper for critical intellectual content.

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**Data availability** Raw data for this article is available.

## Declarations

**Conflict of Interest** The authors declare that they have no competing interests.

**Ethics Approval and Consent to Participate** This study obtained ethical clearance from the Ethical Review Committee of the Kwara state Ministry of Health, Nigeria. Written and informed consent was obtained from children's parents/guardians before enrolling them in this study.

Inclusion criteria were the provision of a signed informed consent and no reported chronic or acute illness except malaria. Exclusion criteria included those who were unwilling to participate in the study.

**Consent for Publication** All authors read and approved the final draft of the paper.

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