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# Evaluation of the Bacterial Profile and Pathogenicity in Concomitant Bacteremia with Malaria among Children in Ekiti State

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AOO did the study design and wrote the protocol. Authors KBD and OOB did the statistical analysis and literature searches while analyses of study was by authors KBD, AOO and OOB. All authors read and approved the final manuscript.*

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## ABSTRACT

Bacteremia concomitant with malaria remain one of the leading causes of mortality and morbidity among children in Africa and the relationship between the two remains unclear. Therefore this study was carried out to determine if malaria predisposes children to bacteremia and the pathogenicity of bacterial isolates in bacteremia concomitant with malaria. A total of 34 bacteria strains belonging to 4 genera were isolated out of which 44.1% were *E. coli,* 29.4% were *Staphylococcus aureus*, 17.7% were *Pseudomonas aeruginosa* and 8.8% were *Salmonella typhi.* Fifty one (27.4%) of the children tested positive for malaria out of which fourteen (7.5%) had concomitant bacteremia and malaria, Thirty seven (19.9%) had malaria only while 20 (10.8%) had bacteremia only. *Escherichia coli* was the most common organisms found in bacteremia concomitant with malaria while *Pseudomonas aeruginosa* was the least with 8 (23.5%) and 1 (2.9%) cases of occurrence respectively. Bacteremia in concomitant with malaria was common in the age group 0-5 years with

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a prevalence of 12 (11.8%). *LasB* and *PVL* genes were detected in all (100%) of the selected *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. The *stx1* and *eae* genes were also present in 50% of the selected *E. coli.* In conclusion, the results show that there is a weak association between bacteremia and malaria in the study area and that malaria did not predispose the children to bacteremia. The ability of the bacteria to invade and survive in the blood is due to the presence of some virulence genes and not malaria parasites.

*Keywords: Bacteremia; malaria; children; concomitant.*

## 1. INTRODUCTION

Invasive bacterial disease and malaria remain the two leading causes of paediatric mortality and morbidity in Africa [1,2]. Over time there has been a piecemeal accumulation of data indicating that children with plasmodium malaria are at risk of invasive bacterial infection however it remains unclear whether malaria infection is a risk factor for invasive bacterial disease [3]. One of the risk factors to develop invasive bacterial disease in Africa is *Plasmodium falciparum* [4]. Those with bacteremia and malaria co-infection have a higher case fatality compared to those with malaria infection only [5]. In malaria endemic areas, 11% of the children admitted with fever are found to have bacteremia and Twelve percent (12%) of those children die because malaria was over diagnosed at the expense of other causes of fever. 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 [2].

Invasive bacterial infections have been associated with high mortality in children with severe malaria in Sub-Saharan Africa [6]. Recently, malaria has been shown to strongly predispose children in malaria-endemic areas to bacteremia [7]. Both malaria and bacterial infections constitute an enormous burden for the under-resourced African health facilities. They represent the principal causes of admission and together account for more than half of all paediatric in-hospital deaths. Both infections predominantly affect young children, who also have the highest risk of dying. Recent studies have shown the existence of concomitant bacterial infections in severe malaria patients, which complicate the manifestation of malaria, thereby confusing diagnosis and chemotherapy especially where they are relatively unknown [3,2].

Differentiation of the clinical presentation of malaria from bacterial sepsis may be difficult leading to a wide underestimation of the role of bacteraemia in causing morbidity and mortality among children in Sub-Saharan Africa. The contribution of bacteremia concomitant with malaria to morbidity and mortality among people in Africa has been a subject of global health interest. Hence this study was carried out to determine if malaria predisposes children to bacteremia.

## 2. MATERIALS AND METHODS

### 2.1 Ethical Consideration, Questionnaire and Informed Consent

The ethical clearance for this research was given by Federal Teaching Hospital ethical committee after due process has being followed. Verbal and written informed consent was administered to the consented subjects to obtain necessary information before collection of samples.

### 2.2 Study Location and Population

Samples were collected from children of 15years or less visiting the Federal Teaching Hospital, (formerly known as Federal Medical Centre) Ido Ekiti, in ido-Osi local Government, Ekiti state between July 2014 and August 2015. Federal Teaching Hospital is a tertiary institution and a major referral centre in Ekiti state and environs.

### 2.3 Collection and Processing of Samples

Venous blood samples of 186 children presented for malaria were collected. About 6 mls Blood samples were cultured on Brain Heart Infusion broth and Thioglycolate broth for the isolation of aerobic and anaerobic bacteria. Isolates were subcultured on Chocolate and MacConkey agar at 37℃ for 24 hours and were further characterised using colonial morphology, Gram reaction and biochemical tests. Identification was done using Bergey's manual of systematic bacteriology. Thick and thin blood films were stained using Giemsa staining techniques to determine the presence and the species of malaria parasites present in the blood samples respectively and were carried out according to

Cheesbrough [8]. Multiplex Polymerase Chain Reaction (PCR) was used to determine the presence of various virulence genes using the procedure according to Leck and Brent [9].

## 3. RESULTS

Out of the 186 blood samples examined, ninety two (49.5%) were males while 94 (50.5%) were females. One hundred and two (54.8%) were in the age group 0-5 years, 47 (25.3%) were in the age of 6-10 years. Those in the age group of 11-15 years had a frequency of distribution totalling 37 (19.9%) (Table 1). A total of 34 bacteria strains belonging to 4 genera were isolated out of which 44.1% were *E. coli,* 29.4% were *Staphylococcus aureus*, 17.7% were *Pseudomonas aeruginosa* and 8.8% were *Salmonella typhi* (Table 2). Fifty one (27.4%) of the children tested positive for malaria parasites. Eleven (21.6%) of the blood samples had 1-10 parasites per 100 field as seen in the thick film under the microscope and was recorded as scanty (+), 15 (29.4%) had 11-100 parasites per hundred fields and was recorded as mild (++) while 25 (49.0%) had about 1-10 parasites per field and was recorded as severe (+++) (Table 3). All the malaria parasites were *Plasmodium falciparum* as observed through the thin film (Table 4).

Fourteen (7.5%) of the children had concomitant bacteremia and malaria, 37 (19.9%) had malaria only, while 20 (10.8%) had bacteremia only (Table 5). *Escherichia coli* was the most common organisms found in concomitant bacteremia with malaria with 8 (23.5%), followed by *Staphylococcus aureus* with 5 (14.7%) with only 1 (2.9%) case of *Pseudomonas aeruginosa*. *Salmonella typhi* was not found in concomitant bacteremia and malaria. Among those with bacteremia only, 7 (20.6%) of the organisms isolated were *E. coli*, 5 (14.7%) were *Staphylococcus aureus*, 3 (8.8%) were *Salmonella typhi*, while 5 (14.7%) were *Pseudomonas aeruginosa* (Table 6). Bacteremia in concomitant with malaria was common in the age group 0-5 years with 12 (11.8%). Bacteremia only and malaria only was also common in the same age group with 15 (14.7%) and 30 (29.4%) cases respectively. The age group 6-10 years had malaria concomitant with bacteremia at a prevalence rate of 2.1%. They also have the prevalence rate of malaria only at 6.4% and a prevalence rate of bacteremia only at 8.5%. The age group 11-15 years had the least case occurence of bacteremia concomitant with malaria with 1 (2.7%), 4 (10.8%) of them had malaria only and 1 (2.7%) had bacteremia only as shown in Table 7. The statistical analysis showed that the relationship between the age group 0-5 years and both infections is statistically significant as p value is less than 0.05 (p value = 0.0394, $X^2$ =5.14, df = 2)

Among the male children, 21 (22.8%) had malaria only, 15 (16.3%) had bacteremia only while 5 (5.4%) had bacteremia concomitant with malaria. Also, 30 (31.9%) of the female children had malaria only, 19 (20.2%) had bacteremia only and 9 (9.6%) had bacteremia concomitant with malaria as shown in Table 8. Infections were more common among females than males. This is not statistically significant as p value is greater than 0.05 (p value 0.073, $x^2$ =9.55, df = 2).

**Table 1. Demographic data of children examined for bacteremia concomitant with malaria**

| Age (yrs) | Male (n=92) (%) | Female (n=94) (%) | Total (%) |
|---|---|---|---|
| 0-5 | 44 (47.8) | 58 (61.7) | 102 (54.8) |
| 6-10 | 28 (30.4) | 19 (20.2) | 47 (25.3) |
| 11-15 | 20 (21.7) | 17 (18.1) | 37 (19.9) |
| Total | 92 (100) | 94 (100) | 186 (100) |

**Table 2. Profile of bacteria isolated from children examined for bacteremia concomitant with malaria**

| Bacteria | Occurence | % |
|---|---|---|
| *E. coli* | 15 | 44.1 |
| *Staphylococcus aureus* | 10 | 29.4 |
| *Pseudomonas aeruginosa* | 6 | 17.7 |
| *Salmonella typhi* | 3 | 8.8 |
| Total | 34 | 100 |

**Table 3. Distribution of *Plasmodium falciparum* parasites in thick blood films of children examined for bacteremia concomitant with malaria**

| Status | Frequency of distribution | No |
|---|---|---|
| Scanty | + = 1–10 per 100 thick fields | 11 (21.6%) |
| Mild | ++ = 11-100 per 100 thick fields | 15 (29.4%) |
| Severe | +++ = 1–10 per thick field | 25 (49.0%) |
| | Total | 51 |

**Table 4. Occurence of *Plasmodium* sp in thin blood films of children examined for bacteremia concomitant with malaria**

| Early forms of malaria parasite | Shapes of parasites | Types of *Plasmodium* sp |
|---|---|---|
| Gametocytes | Banana shaped | *Plasmodium falciparum* |
| Schizonts | Usually with 2 or 4 merozoites and pigment | *Plasmodium falciparum* |
| Trophozoites | Rings , with double chromatin dots | *Plasmodium falciparum* |

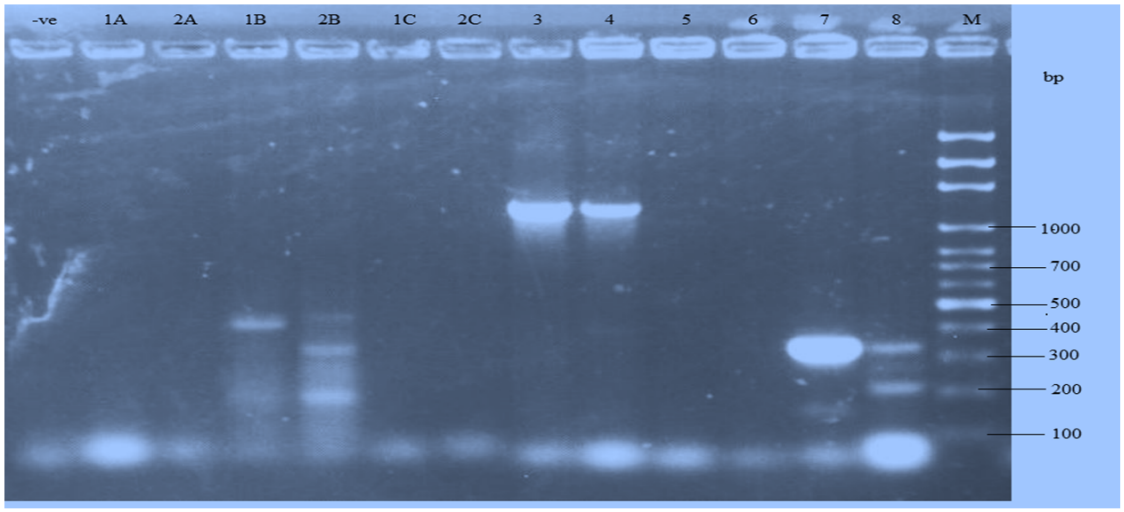


**Fig. 1. Detection of virulence genes in bacteria isolated from children examined for bacteremia concomitant with malaria**

*Key: Lane 1A represents E. coli (from bacteremia only, stx2 gene absent), Lane 2A represents E. coli (from bacteremia only, eae gene absent), Lane 1B represents E. coli (from bacteremia only, bands showing the presence of stx1 gene), Lane 2B represents E. coli (from bacteremia concomitant with malaria, bands showing the presence of eae gene), Lane 1C represents E. coli (from bacteremia concomitant with malaria, stx1 gene absent), Lane 2C represents E. coli (from bacteremia concomitant with malaria, stx2 gene absent), Lane 3 represents Pseudomonas aeruginosa (from bacteremia only, bands showing the presence of lasB gene), Lane 4 represents Pseudomonas aeruginosa (from bacteremia with concomitant malaria, showing the presence of lasB gene) Lane 5 represents Salmonella typhi (bacteremia only, invA gene absent), Lane 6 represents Salmonella typhi (bacteremia only, invA gene absent), Lane 7 represents Staphylococcus aureus (from bacteremia concomitant with malaria, bands showing the presence of PVL gene) Lane 8 represents Staphylococcus aureus (from bacteremia only, bands showing the presence of PVL gene)*

**Table 5. Prevalence of bacteremia and malaria infections in children examined for bacteremia concomitant with malaria**

| Type of infections | Occurence | % |
|---|---|---|
| Bacteremia only | 20 | 10.8 |
| Bacteremia concomitant with malaria | 14 | 7.5 |
| Malaria only | 37 | 19.9 |
| Negative | 115 | 61.8 |
| Total | 186 | 100 |

In the detection of virulence gene using polymerase chain reaction, the bands in lane 1B shows the presence of the gene *stx*1 of size 500 bp in *E. coli* isolated from patients with bacteremia only and bands in lane 2B shows the presence of *eae* gene of size 500 bp in *E. coli* from patient with bacteremia concomitant with malaria. The bands in lane 3 and 4 shows the presence of *lasB* gene of about size 1000 bp in *Pseudomonas aeruginosa* isolated from cases of bacteremia only and bacteremia concomitant with malaria respectively. Also the bands in 7 and 8 shows the presence PVL gene of about size 500 bp in *Staphylococcus aureus* from bacteremia only and bacteremia concomitant with malaria respectively.

**Table 6. Bacterial profile of the blood of children examined for bacteremia concomitant with malaria**

| Organism | Bacteremia concomitant with malaria | Bacteremia only | Total | $X^2$ | df | P value |
|---|---|---|---|---|---|---|
| *E. coli* (n=15) | 8 (23.5%) | 7 (20.6%) | 15 | | | |
| *Staphlococcus aureus* (n=10) | 5 (14.7%) | 5 (14.7%) | 10 | | | |
| *Pseudomonas aeruginosa* (n=6) | 1 (2.9%) | 5 (14.7%) | 6 | 6.591 | 3 | 0.1013 |
| *Salmonella typhi* (n=3) | 0 (0.0%) | 3 (8.8%) | 3 | | | |
| Total | 14 | 20 | 34 | | | |

**Table 7. Prevalence of malaria and bacteremia in children examined for bacteremia concomitant with malaria in relation to age**

| Age (yrs) | Malaria concomitant with bacteremia | Malaria only | Bacteremia only | Total | $X^2$ | Df | P value |
|---|---|---|---|---|---|---|---|
| 0-5 (n=102) | 12 (11.8%) | 30 (29.4%) | 15 (14.7%) | 57 (55.8%) | | | |
| 6-10 (n=47) | 1 (2.1%) | 3 (6.4%) | 4 (8.5%) | 8 (17.0%) | 5.14 | 2 | 0.0394 |
| 11-15 (n=37) | 1 (2.7%) | 4 (10.8%) | 1 (2.7%) | 6 (16.2%) | | | |
| Total | 14 | 37 | 20 | 71 | | | |

**Table 8. Prevalence of malaria and bacteremia in children examined for bacteremia concomitant with malaria in relation to sex**

| Infections | Male (n=92) | Female (n=94) | Total (n=186) | $X^2$ | Df | P value |
|---|---|---|---|---|---|---|
| Malaria only | 21 (22.8%) | 30 (31.9%) | 51 (27.4%) | | | |
| Bacteremia only | 9 (9.8%) | 11 (11.7%) | 20 (10.8%) | 9.55 | 2 | 0.053 |
| Malaria concomitant bacteremia | 5 (5.4%) | 9 (9.6%) | 14 (7.5%) | | | |
| Negative | 57 (62.0%) | 36 (38.3%) | 87 (46.8%) | | | |
| Total | 92 (49.5%) | 94 (50.5%) | 186 (100%) | | | |

## 4. DISCUSSION

The relationship between malaria and bacteremia is important to understand. In this study 7.5% of the children examined had bacteremia concomitant with malaria. The relationship between bacteremia and malaria has been reported in many studies, however in this study there is a weak association between the two infections. The presence of malaria in this study does not appear to show any significant association with bacteremia. This is similar to the study carried out by Christopher and Onaiwu [10] in Benin City where the prevalence of bacteremia concomitant with malaria was 7.8% and it was concluded that there was a weak association between bacteremia and malaria. Also the prevalence of bacteremia concomitant with malaria in a research carried out by Were et al. (2013) in Kenya was 11.7%. The detection of the virulence genes both in cases of bacteremia only and bacteremia concomitant with malaria confirms that malaria was not responsible for the presence of bacteria in the blood. The effacement attachment (*eae*) genes were detected in *E. coli* isolated from cases of bacteremia only and bacteremia concomitant with malaria. This shows that the incidence of concomitant *E. coli* bacteremia was not caused by malaria parasites. The presence of the virulence genes in the bacteria was responsible for their transfer into the blood. Even in the absence of malaria as seen in cases of bacteremia only, the genes were still detected in the bacteria which further confirmed that malaria has nothing to do with the presence of bacteria in the blood. The *eae* gene which was present in 50% of the selected *E. coli* (Fig. 1) in this study has being reported to be necessary for intimate attachment to microvillus effacement and epithelial cells of the intestines which impair gut barrier function and facilitate transfer of the bacteria into the blood stream [1].

The presence of panton-valentin leukocidine (*pvl*) gene in both cases of bacteremia only and bacteremia concomitant with malaria also shows that malaria infection has nothing to do with the presence of *Staphylococcus aureus* in the blood and that the *PVL* gene could be responsible for its invasion. In this study 100% of the selected *Staphylococcus aureus* carry the panton-valentin leukocidine (*PVL*) gene. Wang et al. [11] reported that with the presence of the *PVL* gene, *Staphylococcus aureus* can evade the host immune response by secreting anti-opsonizing proteins which prevent phagocytosis by neutrophils. The *PVL* gene can cause tissue necrosis and leukocyte destruction which favours its translocation into the blood.

The *pseudomonas aeruginosa* from cases of bacteremia only and that from cases of bacteremia concomitant with malaria both habour the *lasB* gene which is believed to be one of the most important protease in the bacteria and it has been reported to promote its development within the infected host and also interfere with the host immune system. This shows that the *LasB* gene in *Pseudomonas aeruginosa* was responsible for its presence in the blood and not malaria as reported by other studies.

As shown in this study *Salmonella typhi* was not found in concomitant bacteremia and malaria. This further confirms that malaria does not predispose children to bacteremia, because even in the absence of malaria the bacteria were still isolated from the blood. Strains of *Salmonella typhi* possess a number of virulence factors that contributes to its ability to enter into the blood. However the *invA* gene tested in this study, which was reported to be one of the genes responsible for its invasion was absent.

In this study, the prevalence of bacteremia concomitant with malaria was highest in young children within the age group 0-5 years with a prevalence of 11.8%. Bacteremia only was also common within the same age group with a prevalence of 14.7%. This may be due to the fact that children under five years are more vulnerable due to factors such as low immune system, gastric acidity and immaturity of gut lymphoid tissue. Evans et al. [12] described that bacteremia concomitant with malaria affects younger children more often and severely than older children.

Similarly, children less than five years of age have being discovered to be at the highest risk of malaria [13]. It is believed that above the age of 5 years, the prevalence of malaria tends to decrease [14]. Thirty seven (19.9%) of the children in this study had malaria, out of which 30 (29.4%) were of age group 0-5 years. This may be the reason for the decline in the prevalence of malaria observed in ages >5 years old in this study. This is similar to the research carried out by Christopher and Onaiwu [10] in Benin, where malaria accounts for 26.2% of the infections in children and also Milicent and Gabriel [15] in Kaduna state in their research reported a figure of 35.7%.

## 5. CONCLUSION

The incidence of bacteremia concomitant with malaria is not disputed in this study, however the results shows that there is a weak association between malaria and bacteremia and that malaria does not appear to predispose children to bacteremia in the study area. The ability of the bacteria to invade and survive in the blood was due to the presence of some virulence genes and not malaria. The incidence of bacteremia concomitant with malaria in children might be a mere coincidence.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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