Original Article

Aetiologic Agents of Fevers of Unknown Origin among Patients in Benin City, Nigeria

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Abstract

Background: **Malaria parasitaemia is an important predictor of bacteremia, concomitant invasive bacterial infections and malaria parasitaemia are strongly associated with death.**

Methods: **Blood samples were collected from 500 patients (281 males and 219 females) with fevers of unknown origin. The blood samples were processed to diagnose malaria and bacterial septicaemia using standard microbiological techniques.**

Results: **The prevalence of concomitant bacterial septicaemia and malaria parasitaemia was 7.8%. The prevalence of malaria parasitaemia alone (26.2%) was significantly ($P < 0.0001$) higher than that of bacterial septicaemia (13%). Patients 61 years old and older had higher prevalences of malaria parasitaemia, bacterial septicaemia, and concomitant infections. The most prevalent organism causing bacterial septicaemia were of the *Klebsiella* species, while ceftriaxone and ceftazidime were the most effective antibacterial agents.**

Conclusion: **Overall prevalence of malaria parasitaemia, septicaemia and concomitant malaria parasitaemia, and bacterial septicaemia were 26.2%, 13%, and 7.8%, respectively. Bacteria from the *Klebsiella* species were the most common bacteria causing septicaemia. Although ceftriaxone and ceftazidime are the recommended treatments, there is need for urgent treatment of concomitant infections due to their poor prognosis.**

Keywords: *malaria, bacterial septicaemia, prevalence*

Introduction

Malaria remains one of the major threats to human health. Nearly 2 billion people are at risk and it is likely that more than 1 million die of the disease annually (1). Malaria is by far the most devastating, acute febrile illness in humans, and it is caused by parasitic protozoa of the genus *Plasmodium* (2). Severe malaria can be clinically indistinguishable from other common illnesses, including pneumonia, meningitis, and sepsis (3). Malaria parasitaemia, among other factors, has been reported to be an important predictor of bacteremia in children residing in malaria endemic areas (4). A number of studies have shown evidence of concomitant bacterial and malarial infections (2,4,5).

Considering that malaria has similar clinical manifestations with other systemic infections (bacterial septicaemia), it is plausible that misdiagnosis can occur. It has been reported that concomitant invasive bacterial infections and malaria parasitaemia are strongly associated with death (3). However, most studies, including those from Nigeria, have looked at bacterial septicaemia in patients with malaria or presenting with symptoms of malaria (4,5). This may not give an accurate picture of the prevalence of concomitant bacteremia and malaria parasitaemia. Against this background, this study aimed to determine the prevalence of malaria parasitaemia, bacterial septicaemia and concomitant bacteremia, and malaria parasitaemia among patients presenting with fevers of unknown origin. In addition, the aetiologic agents of bacterial septicaemia and their susceptibility profiles were determined.

Materials and Methods

Study population and sample collection

The study recruited 500 patients with fevers of unknown origin. The patients either had fever for one week in the hospital or complained of fever lasting for three weeks at outpatient clinics without finding the cause. The study population consisted of 281 males and 219 females, with ages ranging from 1 day to 84 years. Patients either visited one of various outpatient clinics or were

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admitted to the University of Benin Teaching Hospital or Central Hospital, Benin City, Nigeria. Prior to specimen collection, verbal informed consent was obtained from all patients, or in the case of children, from their parents. The Ethical Committee of both health institutions approved the protocol for this study.

Processing of blood culture

Blood samples were collected from each patient, dispensed into ethylene diamine tetra-acetic acid (EDTA) containers and blood culture bottles (glucose broth and thioglycolate broth) and incubated for a maximum of seven days. Bottles with signs of growth, such as turbidity, haemolysis, clot formation, gas production and/or the cotton ball effect were subcultured on chocolate, blood, and MacConkey agar plates. The chocolate agar plates were incubated in candle jars, and the blood and MacConkey agar plates were incubated aerobically. Significant bacterial isolates were identified by standard techniques (6). A disc susceptibility test was performed using the British Standard for Antimicrobial Chemotherapy (BSAC) method (7).

Detection of malaria parasites

Malaria parasites were detected using standard laboratory techniques (8). The EDTA blood sample was mixed, and a drop was used to make a thick blood film. This was allowed to air dry and then was flooded with 10% Giemsa stain and allowed to act for 10 minutes. It was washed with tap water, the back was wiped and the slide was allowed to drain and dry. A drop of oil immersion was placed on the stained, dried film and was examined under the microscope using 100× objective. The study examined 200 fields. Presence of any stage of malaria parasites was taken as positive, and absence was taken as negative.

Statistical analysis

The data were analyzed using the statistical software INSTAT® (Graph Pad Software Inc, La Jolla, CA, USA). Differences in proportion were analyzed with a chi-square (χ^2) test, and a P value of < 0.05 was considered significant at the 95% confidence interval.

Results

Of the 500 patients screened, 65 (13.0%) had bacterial septicaemia, 131 (26.2%) had malaria parasitaemia, and 39 (7.8%) were positive for both. Males had a higher prevalence of malaria parasitaemia (27.8%) and bacterial septicaemia (14.2%) compared with females, although the difference was statistically insignificant (malaria $\chi^2 = 0.632$, df = 1, $P = 0.427$; septicaemia $\chi^2 = 0.634$, df = 1, $P = 0.426$) (Table 1). Similarly, gender did not affect the prevalence of concomitant bacterial septicaemia and malaria parasitaemia ($P = 0.327$).

Age of the subjects affected the prevalence of malaria parasitaemia ($\chi^2 = 15.237$, df = 6, $P = 0.0185$), bacterial septicaemia ($\chi^2 = 13.828$, df = 6, $P = 0.0316$) and concomitant infections ($\chi^2 = 114.48$, df = 6, $P < 0.0001$). Patients aged 50–60 years old had the highest prevalence of malaria parasitaemia and bacterial septicaemia, and patients > 61-years-old had the highest prevalence of concomitant infections (Table 2).

The study recovered 104 bacterial isolates, 61 (58.7%) from males and 43 (41.4%) from females (Table 3). Generally, and among males in particular, bacteria of the *Klebsiella* species were the most prevalent bacteria causing septicaemia, followed by *Staphylococcus aureus*. However, *Staphyloccus aureus* was the most prevalent bacteria causing septicaemia among females. *Citrobacter freundii* and *Providencia* species were not recovered from males, while *Alcaligenes* species, *Streptococcus pneumoniae* and coagulase negative *Staphylococci* were not recovered from females.

Ceftazidime, ceftriaxone, amoxicillin-cluvalanate, and ciprofloxacin showed good antibiotic activity against the bacterial isolates (Table 4), while cotrimoxazole, tetracycline, and chloramphenicol showed low activity against

Table 1: Prevalence of malaria parasitaemia and septicaemia in febrile patients

Gender	Number tested	Number with malaria parasitaemia (%)	Number with bacterial septicaemia (%)	Number with mixed infection (%)	Number of patients without infection (%)
Male	281	78 (27.8)	40 (14.2)	19 (6.8)	144 (51.2)
Female	219	53 (24.2)	25 (11.4)	20 (9.1)	121 (55.3)
Total	500	131 (26.2)	65 (13.0)	39 (7.8)	265 (53.0)

them. Erythromycin showed no activity against the Gram-positive isolates.

Discussion

It has been reported that bacteremia exacerbates the severity of malaria in African children, and bacteremia is a common cause of childhood illness in children presenting with malaria (4,9). The majority of reports have looked at concomitant bacteremia with malaria parasitaemia among children with malaria. Perhaps this is because fever, the most prominent symptom of malaria, is not specific. To our knowledge, this is the first study that looks at concomitant bacterial septicaemia and malaria parasitaemia among patients with fevers of unknown origin.

The patients with infections, malaria parasitaemia accounted for 26.2%, bacterial septicaemia for 13.0%, and mixed malaria and bacterial septicaemia for 7.8%. In this study, the prevalence of malaria parasitaemia was significantly higher than that of bacterial septicaemia ($P < 0.0001$). This clearly indicates that malaria parasitaemia is mostly responsible for fevers of unknown origin in this locality, as this is a malaria endemic region (3). The prevalence of

Table 2: Age distribution of malaria parasitaemia and septicaemia in febrile patients

Age (years)	Number of patients tested	Number of patients with malaria parasitaemia (%)	Number of patients with bacterial septicemia (%)	Number of patients with mixed malaria parasitaemia and bacterial septicaemia (%)	Number of patients without infection (%)
0–10	287	89 (31.0)	43 (15.0)	16 (5.6)	139 (48.4)
11–20	88	17 (19.3)	6 (6.8)	4 (4.5)	61 (69.3)
21–30	43	8 (18.6)	3 (7.0)	0 (0.0)	32 (74.4)
31–40	26	4 (15.4)	3 (11.5)	0 (0.0)	19 (73.1)
41–50	17	5 (41.2)	0 (0.0)	2 (11.8)	10 (58.8)
51–60	10	5 (50.0)	2 (20.0)	0 (0.0)	3 (30.0)
> 61	29	3 (10.3)	8 (27.6)	17 (58.6)	1 (3.4)
Total	500	131 (26.2)	65 (13.0)	39 (7.8)	265 (53.0)

Table 3: Prevalence of bacterial isolates in patients with septicaemia

Organisms	Number of isolates (%) from males	Number of isolates (%) from female	Total number of isolates (%)
Klebsiella spp	27 (44.3)	9 (20.9)	36 (34.6)
Enterobacter spp	3 (4.9)	5 (11.6)	8 (7.7)
Escherichia coli	3 (4.9)	6 (14.0)	9 (8.7)
Citrobacter freundii	0 (0.0)	2 (4.7)	2 (1.9)
Proteus vulgaris	3 (4.9)	2 (4.7)	5 (4.8)
Proteus mirabilis	6 (9.8)	4 (9.3)	10 (9.6)
Acinetobacter spp	3 (4.9)	4 (9.3)	7 (6.7)
Providencia spp	0 (0.0)	1 (2.3)	1 (1.0)
Alcaligenes spp	2 (3.3)	0 (0.0)	2 (2.0)
Staphylococcus aureus	10 (16.4)	10 (23.3)	20 (19.2)
Coagulase negative *staph*	3 (4.9)	0 (0.0)	3 (2.9)
Streptococcus pneumoniae	1 (1.6)	0 (0.0)	1 (1.0)
Total	61 (58.7)	43 (41.4)	104 (100)

malaria reported in this study (26.2%) was higher than previously reported by the Orogade et al. and Peters et al. studies (8.25% and 12%, respectively) (10,11) but lower than that reported by Ehart et al. (12), who reported a prevalence of 52.76%. This difference could be due to geographic location, type of subjects and age of subjects. The Ehart et al. (12) and Peters et al. (11) studies were carried out in Thailand and Malawi, respectively, and the ages of their study subjects were > 20 and 40 years old, respectively. Orogade's study (10) was conducted in Northern Nigeria with neonates aged 1 day to 6 days. However, in our study, conducted in Benin City, Nigeria, the age range of patients was from 1 day to 84-years-old.

The prevalence of bacterial septicaemia observed in this study is lower than that reported earlier in Nigeria and some other African countries (5,13,14). The prevalence of mixed bacterial and malaria infections was also lower (7.8%) than that previously reported by Were et al. and Ukaga et al. (4,5), which showed a prevalence of 35.2% and 11.7%, respectively. The higher values reported by Were et al. (4) and Ukaga et al. (5) may be because they used patients who had been confirmed to have malaria parasitaemia. In this study, we screened for malaria parasitaemia and bacterial septicaemia among patients with fevers of unknown origin.

Gender did not significantly affect the prevalence of malaria parasitaemia, bacterial septicaemia or concomitant infections (P = 0.3961, P = 0.9024 and P = 0.327, respectively). This finding in terms of bacterial septicaemia

Table 4: Susceptibility profile of bacterial agents of septicaemia

Organisms	Antibacterial agents (µg/disc) (*n*/%)										
	CAZ 30 µg	CRO 30 µg	CN 10 µg	AUG 30 g	TE 10 µg	OB 5 µg	SXT 10 µg	C 30 µg	AMX 30 µg	E 5 µg	CIP 5 µg
Klebsiella spp (*n* = 36)	25 (69.4)	28 (77.8)	5 (13.9)	20 (55.0)	1 (2.8)	0 (0.0)	4 (11.1)	1 (30.6)	1 (2.8)	0 (0.0)	28 (77.8)
Escherichia coli (*n* = 8)	6 (66.7)	7 (77.8)	1 (11.1)	7 (77.8)	2 (22.2)	0 (0.0)	1 (11.1)	1 (11.1)	0 (0.0)	0 (0.0)	5 (55.6)
Enterobacter spp (*n* = 9)	3 (37.5)	7 (87.5)	0 (0.0)	6 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (75.4)
Citrobacter freundii (*n* = 2)	2 (100.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)
Proteus vulgaris (*n* = 5)	4 (80.0)	5 (100.0)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	3 (60.0)
Proteus mirabilis (*n* = 10)	4 (40.0)	10 (100.0)	5 (50.0)	5 (50.0)	0 (0.0)	0 (0.0)	2 (20.0)	4 (40.0)	2 (20.0)	0 (0.0)	8 (80.0)
Acinetobacter spp (*n* = 7)	6 (85.7)	6 (85.7)	6 (85.7)	7 (100.0)	1 (14.3)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	0 (0.0)	4 (57.4)
Providencia spp (*n* = 1)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Alcaligenes spp (*n* = 2)	2 (100.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Staphylococcus aureus (*n* = 20)	17 (85.0)	20 (100.0)	13 (65.0)	17 (85.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (35.0)	14 (70.0)	0 (0.0)	15 (75.0)
Coagulase negative staph. (*n* = 3)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (67.7)
Streptococcus pneumoniae (*n* = 1)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)

Abbreviation: CAZ: Ceftraxone; TE: Tetracycline; AMX: Amoxycillin; CRO: Ceftazidime; OB: Cloxacillin; E: Erythromycin; CN: Genticin; SXT: Cotrimoxazole; CIP: Ciprofloxacin; AUG: Cloxacillin/Clavulanate Acid; C: Chloramphenicol.

agrees with that of Ukaga et al. (5) but did not agree with that of Mugalu et al. (14) and Were et al. (4), both of whom reported females as having a higher prevalence of bacterial septicaemia than males. The reason for this difference could be geographic location, as the Mugalu et al. (14) and Were et al. (4) studies were carried out in Uganda and Kenya, respectively. Differences between our study and those of Mugalu et al. (14) and Were et al. (4) also may be explained by the times of the year the studies were carried out, as geographical location and time of the study have been reported to affect the prevalence of septicaemia (19). In terms of malaria, our findings did not agree with those of Incardona et al. (15), who found that males had a significantly higher prevalence of malaria compared to the females. That study reasoned that the high prevalence in males was because males were more likely to be exposed to malaria vector due to farming or forestry activities, such as woodcutting, hunting, and gemstone mining, working with the upper body uncovered and staying outside late at night with no bed-net protection.

In the current study, 53% of samples were negative for malaria parasitaemia and/or bacterial septicaemia. This may be due to viraemia or other infections, such as meningitis and pneumonia, which are clinically indistinguishable from severe malaria, including cerebral malaria (3).

Age significantly (P = 0.0185) affected the prevalence of malaria parasitaemia in the current study. Malaria prevalence decreased with age until age 31–40 years old. Thereafter, the prevalence increased. The effect of age on the prevalence of malaria differs from one study to another. In some studies, the prevalence is highest between 0–5 years and decreases thereafter, a finding consistent with immunity acquired in an exposure-related manner (16). Some researchers reported that age had no effect on the prevalence of malaria (17), while others observed an increase in its prevalence with age (15,18). The higher prevalence of malaria in children older than 5-years-old is attributed to lower background immunity and the environment (17,18). Findings in the current study are in agreement with the fact that immunity to malaria, especially in endemic areas, is reported to increase in an exposure-related manner, and it is believed that above the age of 5-years-old, the prevalence of malaria tends to decrease (16). This may be the reason for the decline in the prevalence of malaria observed in those 0–40 years old in this study. The increase in the prevalence of malaria parasitaemia in those 41 -years-old and older may be due to increased stress occasioned by financial and societal responsibility and an increase in debilitating conditions, such as diabetes, hypertension etc, all of which are capable of lowering immunity and, thus, may be responsible for increased prevalence in this age group. A similar pattern of age prevalence was observed with bacterial septicaemia (P = 0.0316). Prevalence decreased with age until 41–50 years old, after which it increased. These findings agree with previous studies (13,14,19). However, the age range of patients in these studies was 1 day to 15 years old.

The reason adduced for the relationship between malaria parasitaemia, bacterial septicaemia, and age may suffice for the relationship between age and concomitant infections, too. The most common bacteria isolated from neonates in late onset septicaemia were from the *Klebsiella* species. *Staphylococcus aureus* was the most prevalent bacteria causing septicaemia among females. This agrees with the results of Meremikwu et al. (13). In addition, the bacteria species recovered from blood cultures in this study are similar to isolates obtained in other reports (13).

The antibacterial susceptibility pattern of bacterial isolates of septicaemia varies from area to area. This study observed that ceftriaxone and ceftazidime were the most active antibacterial agents. This agrees with previous reports (13,19). The high antibiotic activity of ceftriaxone and ceftazidime may be because they are expensive and given perenterally. Thus, abuse of these antibiotics may not be common. The activity of gentamicin against bacterial agents of septicaemia also varies from one study to another. Some researchers have reported moderate to higher activity (13,19), while others have reported poor activity (20). In the current study, the susceptibility of bacterial isolates of septicaemia to gentamicin was poor to high, depending on the bacterial isolate. Gentamicin is a cheap antibiotic, resulting in abuse, as it is often used as blind therapy without laboratory guidance in empiric therapy of many infections (21). That may explain this result.

Amoxicilin–clavulanate showed good activity against the bacterial isolates, with the exception of the two strains of *Alcaligenes* species and the single isolate of *Streptococcus pneumoniae*, none of which were susceptible. These results are in agreement with the report of Nwadioha et al. (19). In that study, amoxicillin showed poor activity against bacterial isolates with the exception of those from the Providencia species and 70% of the *Staphylococcus aureus* strain. Clavulanic acid is a β–lactamase inhibitor, and its addition to

amoxicillin confers high activity in comparison to amoxicillin alone. This may explain the results for amoxicillin – clavulanate and amoxicillin in this study.

Tetracycline showed poor activity in this study. Long-term use, over the counter sales without prescription, cheap cost, and self-medication have been suggested as possible reasons for bacteria resistance in developing countries (21). This may explain the results for tetracycline in this study. All Gram-positive isolates were resistant to erythromycin and cloxacillin. Antibiotic susceptibility patterns vary with time and location, even within the same country (13). This may explain the difference in susceptibility results for erythromycin and cloxacillin in this study.

Ciprofloxacin activity observed in this study was 55.6–100%. However, ciprofloxacin is contra-indicated in paediatrics (19). This limits its use, making β–lactamase–ceftriaxone and ceftazidime the drugs of choice in paediatric settings. This is important, as the paediatric group form the bulk of this study population.

Conclusion

The prevalence of concomitant bacterial septicaemia and malaria parasitaemia was 7.8%. The prevalence of malaria parasitaemia (26.2%) was significantly higher than that of bacterial septicaemia (13.0%). The predominant bacterial isolate causing septicaemia was the *Klebsiella* species. Ceftriaxone, ceftazidime, and ciprofloxacin were the most active antibacterial agents. Due to the poor prognosis of co-infection, there is need for urgent treatment with effective antibacterial and antimalarial agents.

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Conflict of Interest

None.

Funds

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Authors' Contributions

Conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for the important intellectual content, final approval of the article and statistical expertise: CAE, OIE
Collection and assembly of data: CAE

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