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High Circulation of Malaria and Low Prevalence of Bacteremia in Febrile and Afebrile Children in Northeastern Gabon

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Abstract. The epidemiology of febrile illness etiologies is under-explored in resource-poor settings. Establishing a local repertory of microorganisms circulating in blood of febrile and afebrile people is important for physicians. Blood was collected from 428 febrile and 88 afebrile children in Makokou (Gabon) and analyzed using polymerase chain reaction. *Plasmodium* spp. were the pathogens, which were most detected in febrile children (69.6%; 298/428) and in afebrile children (31.8%; 28/88) ($P < 0.0001$). *Plasmodium falciparum* was the most prevalent species in both febrile and afebrile children (66.8% and 27.3%, respectively). No differences were observed between febrile and afebrile children for *Plasmodium malariae* and *Plasmodium ovale* (8.2% versus 10.2% and 3.3% versus 3.4%, respectively). Triple infection with *P. falciparum*, *P. malariae*, and *P. ovale* was also detected in 1% of febrile children (4/428). Filariasis due to *Mansonella perstans* was detected in 10 febrile patients (2.3%), whereas *Loa loa* was detected in both febrile and afebrile children (1.4% and 2.3%, respectively). Bacterial DNA was detected in only 4.4% (19/428) of febrile children, including 13 (68.4%) who were coinfected with at least one *Plasmodium* species. These were *Haemophilus influenzae* (1.6%, 7/428), *Streptococcus pneumoniae* and *Staphylococcus aureus* (1.2%, 5/428), and *Rickettsia felis* (0.9%, 4/428). *Coxiella burnetii*, *Bartonella* spp., *Borrelia* spp., *Tropheryma whipplei*, *Anaplasma* spp., *Leptospira* spp., *Streptococcus pyogenes*, and *Salmonella* spp. were not detected. This study also highlights the over-prescription and the overuse of antibiotics and antimalarials. Overall, malaria remains a major health problem in Makokou. Malaria control measures must be reconsidered in this region.

INTRODUCTION

In sub-Saharan Africa, where malaria transmission is very high and fever is a common clinical presentation,[1–3] presumptive treatment of fever as malaria has been adopted in many countries.[4] This policy was encouraged by the lack of facilities for malaria diagnosis and the shortage of qualified personnel.[3,5] In Uganda, the evaluation of presumptive diagnostic of malaria showed a high sensitivity but low specificity and positive predictive value. This highlights the fact that malaria has been over-diagnosed in many malaria-endemic countries, including Uganda.[6] In Tanzania, between February 2002 and 2003, a fatality rate of 7.6% was found in 1,571 patients with severe febrile illness, which had been inaccurately treated as malaria.[7] The overdiagnosis of malaria leads to the worsening of underlying diseases which remain untreated and have a poor prognosis.[6] In 2010, WHO claimed that the decrease in malaria cases and deaths, particularly in Africa, was the result of universal coverage called for by the United Nations Secretary-General.[8] To accurately treat febrile patients and avoid the unnecessary use of hundreds of thousands of artemisinin-based combination therapies (ACT) each year, WHO changed the presumed treatment of malaria by pretreatment diagnosis in 2010.[9] This policy was accompanied by the introduction of a rapid diagnostic test (RDT) for malaria. Because malaria RDTs are easier to use and interpret, provide rapid results, and can be used with very little equipment and training, this has substantiated the hypothesis that many fevers, especially in Africa, are non-malarial. Thus, research on non-malarial febrile illness has become a priority.

Many studies on this topic show that bacterial bloodstream infections are the main cause of non-malarial fever, and that they increase malaria-related mortality from 10.2% in people with malaria alone to 24.1% in people coinfected with malaria/bacteremia.[10] In 2012, of 250 Ugandan children admitted for a presumptive diagnosis of severe malaria, who had been prescribed antimalarial medication by the attending physician and presented negative Giemsa-stained thick blood, 19.1% had bacteremia, mainly due to *Staphylococcus aureus* and non-typhoid *Salmonella*.[11] In 2007 and 2008, of the 870 pediatric and adult febrile admissions to two hospitals in northern Tanzania, 60.7% of them were clinically diagnosed with malaria, but this was the actual cause of fever for only 1.6% of them, whereas acute bacterial zoonoses were diagnosed in 118 (26.2%) of them, including Q fever, leptospirosis, brucellosis, and rickettsioses.[12]

In rural areas of Senegal, molecular studies on the causes of fever were launched in 2008. Since then, it has been well established that *Borrelia crocidurae* may be responsible for up to 25% of febrile illness each year.[13] *Tropheryma whipplei* has been associated with epidemic fever and has been found in 6.4% of non-malarial febrile illness.[14,15] Q fever, *Bartonella quintana*, and rickettsial species have also been identified.[16,17] In 2008, in Libreville (the capital of Gabon), of 418 febrile children admitted to hospital with suspected malaria, 168 were treated as having clinical malaria, but only 95 of them (56.7%) presented positive blood smears for *Plasmodium*

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falciparum, low respiratory tract infections being the main cause of fever.[18] However, in 2015–2016, studies conducted in four cities in southern Gabon reported a variable malaria prevalence of 97.5% (130/134) and 94.8% (55/58) in Lastourville and Fougamou (rural areas), between 54.5% (42/77) and 74.5% (591/793) in Franceville (urban area) and of 68.1% (96/141) in Koulamoutou (semi-urban area).[19,20] Given this spatial and temporal variation, it is important to monitor the etiologies of febrile illness in all regions of Gabon. In Makokou, a semi-urban city of northeastern Gabon, there is a lack of published data regarding this topic. Our aim in this article was to investigate the causes of febrile illnesses among children in this area.

MATERIALS AND METHODS

Ethics statement. This study was approved by the National Committee for Research Ethics (CNER) in Gabon (No. 0020/2015/SG/CNE). A written informed consent signed by the legal guardian or parents was obtained for each child included.

Study design and period and location of study. This is a retrospective study investigating bacteria and parasites circulating in the blood of febrile and afebrile children in Makokou, using molecular tools. Makokou is located in the province of Ogooué-Ivindo, slightly north of the equator (Figure 1), and covered by dense evergreen humid forest. The climate is equatorial, with four alternating seasons: a mainly rainy season (from mid-September to mid-December), a short dry season (from mid-December to mid-March), a short wet season (from mid-March to mid-June), and a mainly dry season (from mid-June to mid-September). In this region, the transmission of malaria is perennial.[21] Medical records and blood samples of children under 15 years, who attended a consultation in the reference hospitals complaining of fever between November 26, 2015 and January 25, 2016, were collected. We obtained permission to explore these data from the CNER in Gabon.

Inclusion criteria for febrile and afebrile children and places of recruitment. Febrile children were recruited in two hospitals in Makokou: The Regional Omar Bongo Ondimba Hospital Center (CHROBOM) and the Makokou Regional Hospital. The eligibility criteria for febrile children were 1) attending a consultation complaining of fever (axillary temperature ≥ 37.5°C) in one of the two aforementioned hospitals mentioned, 2) under the age of 15, 3) resident of Makokou, and 4) informed consent received from parents or legal guardian. The afebrile children were recruited at the same time in a Catholic primary school very close to the CHROBOM hospital by medical staff from the CIRMF (International Medical Research Centre in Franceville, Gabon), who were on mission in Makokou. The first eligibility criterion for afebrile children was to have an axillary temperature < 37.5°C and no health problems during the last month, and the other three criteria were the same as those aforementioned for febrile children. All participants were followed until they were discharged from the hospital.

Sample collection and DNA extraction. Venous blood was collected in EDTA tubes on admission before any antibiotic

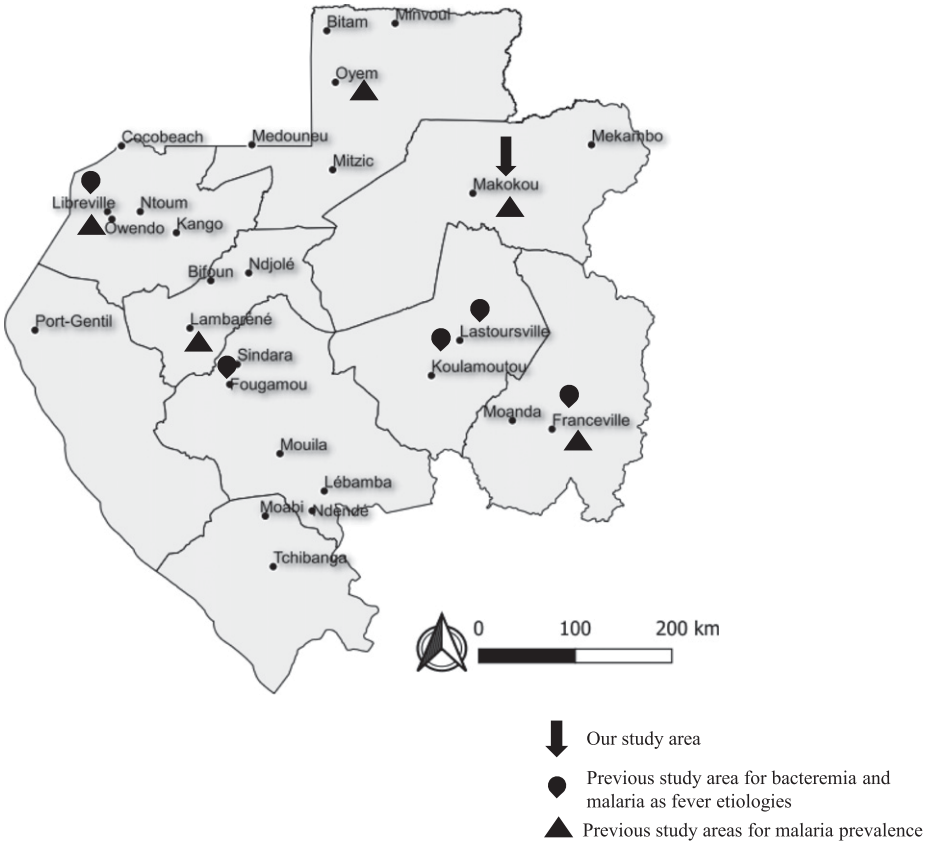

FIGURE 1. Current and previous study areas. The area marked with a black arrow is our study area. Circles represent zones having been studied for fever causes. Triangles display areas that have been studied for malaria prevalence only.

or antimalarial prescription by physicians and transported to the CIRMF for storage at –20°C until further analysis. Genomic DNA was extracted from blood in this research center using the EZNA® Blood DNA Tissue Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's protocol described previously.[22] DNA extracts were transferred to the IHU Méditerrannée Infection (Marseille, France) for molecular screening of microorganisms.

Microorganism screening. DNA quality controls were assessed using real-time quantitative PCR (qPCR), targeting β-actin, as previously reported.[23] The samples were then screened for a large range of microorganisms, including parasites (*P. falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, *Mansonella perstans*, and *Loa loa*), fastidious bacteria (*Coxiella burnetii*, *Rickettsia felis*, *Bartonella* spp., *Borrelia* spp., *T. whipplei*, *Anaplasma* spp., and *Leptospira* spp.) and common bacteria (*Streptococcus pneumoniae*, *S. aureus*, *Salmonella* spp., *Haemophilus influenzae*, and *Streptococcus pyogenes*) using qPCR and standard polymerase chain reaction (PCR) coupled with sequencing, if required. The primers and probes used in this study are mentioned in supplemental data (Supplemental Table 1).

Quantitative PCR. For all microorganisms screened, the final reactive volume of 20 μL contained 10 μL of master mix (2×) (Roche Diagnostics GmbH, Mannheim, Germany), 0.5 μL of each primer at 20 μM, 0.5 μL of 5 μM probe, and 5 μL of DNA template. To ensure the reliability of our results, positive controls (either plasmid or genomic DNA of targeted microorganisms) and negative controls (our mix) were included in each PCR run. All amplification cycles were performed using the CFX96 Real-Time system (Bio-Rad Laboratories, Foster City, CA) as follows: a first step of 50°C for 2 minutes, followed by initial denaturation step at 95°C for 5 minutes and 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds.

Standard PCR and sequencing. Standard PCR coupled with Sanger sequencing of intergenic spacer 1 was performed to distinguish filarial species from all positive qPCR samples. The final reactive volume of 25 μL contained 12.5 μL of AmpliTaq gold, 0.75 μL of each primer (20 μM), and 5 μL of DNA template. Amplifications were performed in a Peltier PTC-200 model thermal cycler (MJ Research Inc., Watertown, MA) as follows: a first incubation step at 95°C for 15 minutes, 40 1-minute cycles at 95°C, 30 seconds at 58°C, and 1 minute at 72°C, followed by a final extension for 5 minutes at 72°C. Polymerase chain reaction products were run on agarose gel electrophoresis and results were viewed under UV light using SYBR® safe Gel Stain (Invitrogen, Waltham, MA) present in the agarose gel.

Polymerase chain reaction products were purified using a NucleoFast 96 PCR plate (Macherey-Nagel EURL, Hoerdt, France) and sequenced using the BigDye® terminator V3.1 Cycle sequencing kit (Perkin Elmer Applied Biosystems, Foster City, CA) with 3130xl Genetic Analyzer equipment (Applied Biosystems™). The sequences obtained were processed using ChromasPro software (version 1.7.7 copyright© 2003–2015 Pty Ltd., Tewantin, Australia) and compared with filariae sequences existing in GenBank, with blast application.

Statistical analysis. The results were processed using SAS software version 9.4 (SAS institute Inc., Cary, NC). The chi-squared test and Fisher's exact test were used to compare frequencies. Two-sided *P*-values were set at 0.05 for statistical significance.

RESULTS

Demographic characteristics. Overall, 428 febrile and 88 afebrile children were included. The mean age of all children included was 50 (±43.5) months. Fifty-one per cent of participants (263/516) were male, giving a sex ratio of 1.03. Overall, 66.9% (345/516) of children had PCR-detectable microorganism in their blood, 74% (317/428) were febrile, and 31.8% afebrile (28/88; $P < 0.0001$).

Parasites. Malaria parasites were the most common microorganisms (63.2%; 326/516). The prevalence of malaria in febrile children (69.6%; 298/428) was significantly higher than in afebrile children (31.8%; 28/88) ($P < 0.0001$). *Plasmodium falciparum* was the most frequently identified *Plasmodium* species, with an overall prevalence of 60.1% (310/516) and 95.1% (310/326) of all positive samples for *Plasmodium* spp. (Table 1, Figure 2). Its prevalence was significantly higher (66.8%; 286/428) in febrile children than in afebrile children (27.3%, 24/88; $P < 0.0001$). Furthermore, the mean cycle threshold (Ct) value for *P. falciparum* was significantly lower in febrile children (24.5 ± 5.1) than in afebrile children (28.2 ± 2.7; $P < 0.0001$), consistent with higher parasitemia in febrile children (Figure 3). The overall prevalence of *P. malariae* was 8.5% (44/516), accounting for 13.5% (44/326) of all positive

TABLE 1
Prevalence of screened pathogens in febrile and afebrile children

	Prevalence of detected microorganisms				
	516 children	428 febrile	88 afebrile		
	Positive (%)			*P*-value*	Odds ratio [95% CI]
Bacteria					
Haemophilus influenzae	7 (1.4%)	7 (1.6%)	0	0.609	–
Staphylococcus aureus	5 (1.0%)	5 (1.2%)	0	0.594	–
Streptococcus pneumoniae	5 (1.0%)	5 (1.2%)	0	0.594	–
Rickettsia felis	4 (0.9%)	4 (0.9%)	0	–	–
Parasites					
Plasmodium falciparum	310 (60.1%)	286 (66.8%)	24 (27.3%)	< 0.001	5.703 [3.386; 9.603]
Plasmodium malariae	44 (8.5%)	35 (8.2%)	9 (10.2%)	0.531	0.585 [0.585; 1.340]
Plasmodium ovale	17 (3.3%)	14 (3.3%)	3 (3.4%)	0.999	0.704 [0.184; 2.691]
Mansonella perstans	10 (1.9%)	10 (2.3%)	0	0.224	–
Loa loa	8 (1.6%)	6 (1.4%)	2 (2.3%)	0.63	0.421 [0.075; 2.375]

* $P < 0.05$ (Chi-square or Fisher's exact test).

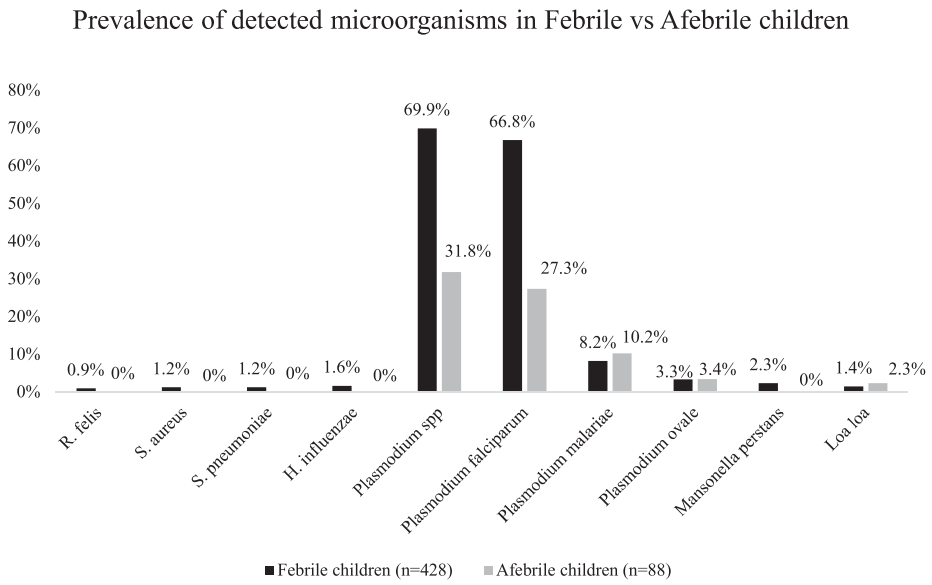

FIGURE 2. Prevalence of detected microorganisms among 428 febrile children (black bars) versus 88 afebrile children (gray bars).

samples for malaria. No significant differences were observed between febrile children (8.2%; 35/428) and children without fever (10.2%; 9/88; P = 0.531). The overall prevalence of *P. ovale* was 3.3% (17/516), representing 5.2% of all positive samples for *Plasmodium* spp. (17/326). No differences were observed between febrile children (3.3%, 14/428) and those with no fever (3.4%, 3/88; P = 0.999).

The overall prevalence of *Loa loa* was 1.6% (8/516). No differences were observed between febrile (1.4%, 6/428) and afebrile children (2.2%, 2/88; P = 0.630). The overall prevalence of *M. perstans* was 1.9% (10/516). *Mansonella perstans* was only detected among febrile children (2.3%, 10/428; P = 0.224).

Bacteremia. Bacteremia with at least one of the screened bacteria was detected in 19 children (19/516; 3.7%). In addition, it was only detected in febrile children (4.4%, 19/428). *Haemophilus influenzae* was the most prevalent bacterium, detected in seven of the 428 febrile children (1.6%). The prevalence of *S. pneumoniae* and *S. aureus* was similar (1.2%, 5/428). Among zoonotic bacteria, only *R. felis* was observed in approximately 1% (4/428) of febrile children (Table 1, Figure 2).

Coinfections. Overall, 12.9% (55/428) of febrile children had mixed infections with at least two microorganisms compared with 10.2% (9/88) of afebrile children (P = 0.499). No coinfection between malaria and bacteria was observed in afebrile children. Coinfection with *P. falciparum* and *P. malariae* was observed in 9.8% (32/326) of all malaria-positive samples (Figure 4) and prevalence was almost the same in febrile (6.1%, 26/428) and afebrile children (6.8%,

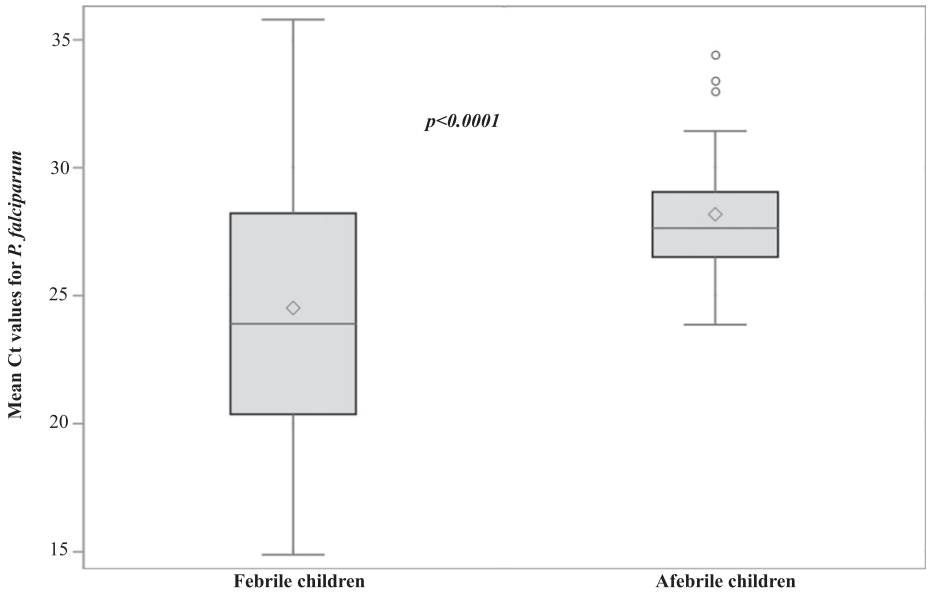

FIGURE 3. Means of Cycle threshold values for *Plasmodium falciparum* based on specific quantitative PCR targeting *PfEMP1* gene in both febrile and afebrile children.

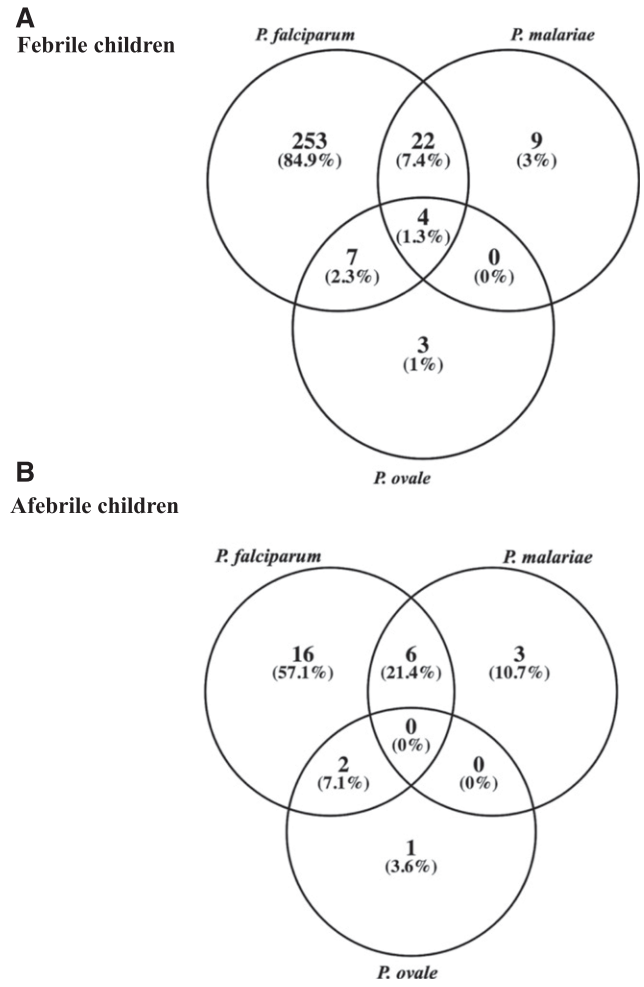

FIGURE 4. Coinfections between *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale* among 298 febrile children (**A**) and 28 afebrile children (**B**).

6/88; $P = 0.792$). Coinfection with *P. falciparum* and *P. ovale* was found in 4% (13/326) of participants with no statistical significance between febrile (2.6%; 11/428) and afebrile (2.3%; 2/88) children ($P = 1$). Coinfection with *P. malariae* and *P. ovale* was only detected in four febrile children (0.8%, 4/428). A triple infection with *P. falciparum*, *P. malariae*, and *P. ovale* was only detected in four febrile children (0.8%, 4/428).

Overall, 3.0% (13/428) of febrile children had coinfections with at least one bacterium and one *Plasmodium* species (Table 2), which represented 68.4% (13/19) of patients with bacteremia and 4.4% (13/326) of patients with malaria due to *P. falciparum*. Two patients were concurrently infected by four microorganisms: one with *P. falciparum*, *H. influenzae*, *S. aureus*, and *S. pneumoniae* and one with *P. falciparum*, *P. malariae*, *P. ovale*, and *M. perstans*. The details of all mixed infections are summarized in Table 2.

Discrepancies between retrospective diagnosis and treatment during consultation. Before the consultation at the hospital, self-medication with antimalarial and antibiotic drugs was reported for 9% (38/428) and 1.7% (7/428) of patients, respectively. At the time of consultation at the hospital, before the molecular diagnosis, antimalarial and antibiotic drugs were prescribed for 62.6% (268) and 34.8% (149) of the 428 patients, respectively.

Of the 298 febrile children with a molecular diagnosis of malaria, 227 received antimalarial drugs (76.2%) and 71 received no treatment for malaria (Figure 5). Of the untreated patients, 50 were infected with *P. falciparum*, seven with *P. malariae*, one with *P. ovale*, 12 with *P. malariae*, and *P. falciparum* and one with the three *Plasmodium* species. At the same time, 41 of the 130 febrile children without malaria (31.5%) received antimalarial drugs (36 quinine-based treatments, four artesunate–amodiaquine, and one artemether–lumefantrine).

Only seven of the 19 patients with bacteremia (36.8%) received antibiotic therapy (Figure 5). Of them, four patients were infected with *H. influenzae*, three received ampicillin and gentamicin, and one received amoxicillin and clavulanic acid. The other three patients received ampicillin and gentamicin: two were infected with *S. aureus* and one with *R. felis*. Of the 12 febrile children with bacteremia who did not receive antibiotics, two were infected with *H. influenzae*, two with *S. aureus*, three with *R. felis*, four with *S. pneumoniae*, and one was diagnosed concomitantly with *S. pneumoniae*, *S. aureus*, and *H. influenzae*. Finally, 34.7% (142/409) of febrile children without a molecular diagnosis of bacteremia received ampicillin (16), amoxicillin (5), amoxicillin/clavulanic acid (4), amoxicillin/clavulanic acid and erythromycin concomitantly (2), ampicillin plus gentamicin (108), amoxicillin

TABLE 2
Coinfections occurred among febrile and afebrile children

Coinfections	428 febrile children positive (%)	88 afebrile children positive (%)	*P*-value
P. falciparum, *P. malariae*, *P. ovale*, and *M. perstans*	1 (0.2)	0	–
P. falciparum, *H. influenzae*, *S. aureus*, and *S. pneumoniae*	1 (0.2)	0	–
P. falciparum, *P. malariae*, and *P. ovale*	3 (0.7)	0	–
P. falciparum, *P. malariae*, and *M. perstans*	1 (0.2)	0	–
P. falciparum, *P. malariae*, and *H. influenzae*	1 (0.2)	0	–
P. falciparum, *P. malariae*, and *L. loa*	1 (0.2)	0	–
P. falciparum and *P. malariae*	18 (4.2)	6 (6.8)	0.792
P. falciparum and *P. ovale*	6 (1.4)	2 (2.3)	1.000
P. falciparum and *M. perstans*	5 (1.2)	0	0.609
P. falciparum and *L. loa*	4 (0.9)	1	1.000
P. falciparum and *Rickettsia felis*	4 (0.9)	0	–
P. falciparum and *S. pneumoniae*	3 (0.7)	0	–
P. falciparum and *S. aureus*	3 (0.7)	0	–
P. falciparum and *H. influenzae*	1 (0.2)	0	–
P. malariae and *M. perstans*	1 (0.2)	0	–

P. falciparum = *Plasmodium falciparum*; *P. malariae* = *Plasmodium malariae*; *P. ovale* = *Plasmodium ovale*; *H. influenzae* = *Haemophilus influenzae*; *M. perstans* = *Mansonella*; *L. loa* = *Loa loa*; *S. aureus* = *Staphylococcus aureus*; *S. pneumoniae* = *Streptococcus pneumoniae*.

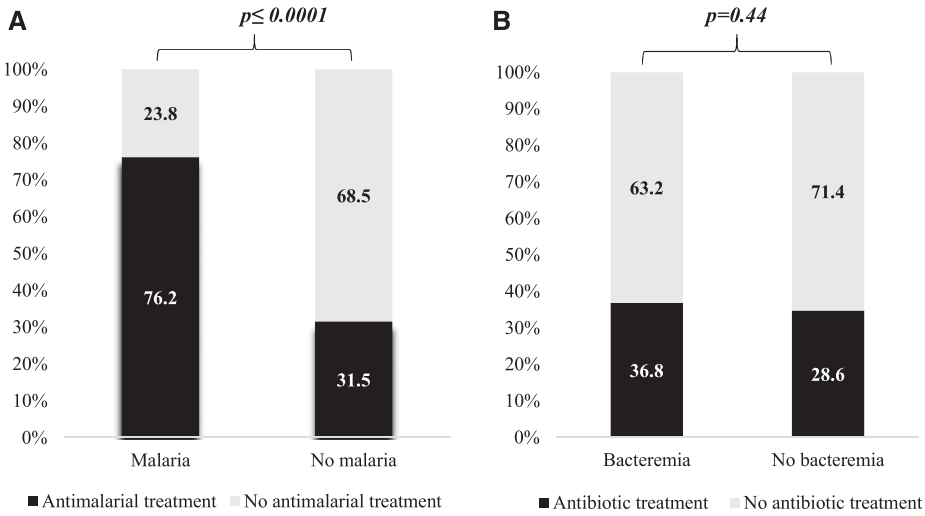

FIGURE 5. Discrepancies between retrospective molecular diagnostic and treatment previously prescribed at the hospital by a physician. (**A**) Discrepancy between retrospective molecular diagnosis of malaria and prescribed antimalarial treatment. (**B**) Discrepancy between retrospective molecular diagnosis of bacteremia and prescribed antibiotic treatment.

plus gentamicin (1), cotrimoxazole (3), erythromycin (2), and gentamicin (1).

DISCUSSION

We conducted a study based on PCR assays targeting specific microorganisms in the blood of febrile and afebrile children in Makokou, a rural city in northeastern Gabon. Our data show that of all screened microorganisms, *Plasmodium* spp. were the most commonly detected in both febrile and afebrile children, with *P. falciparum* being the most prevalent species. The detection of *P. falciparum* was higher than that reported in the same area between 2006 and 2013 (53.6% of 21,337 participants, $P < 0.0001$), although this was obtained using a blood smear test, which is less sensitive than PCR.[24] The comparison between the previous study and the current study must be made with caution as different diagnostic tools have been used. Overall, *Plasmodium* spp. is still detected in a large proportion of children. Malaria remains an important public health concern in Makokou because even if we were not able to ascertain that malaria was the cause of the current fever, studies of mosquitoes feeding on human skin showed that transmission occurs from humans with submicroscopic malaria to mosquitoes.[25,26] Moreover, *P. falciparum* parasitemia was statistically greater in febrile children than in afebrile children. This corroborates the results of previous studies which showed that the induction of fever by *P. falciparum* is dose dependent.[20]

For the first time in Gabon, a triple infection with *P. malariae*, *P. ovale*, and *P. falciparum* was observed in three febrile children. The same triple infection has already been reported using PCR in febrile children in the Central African Republic,[27] in 7% of 274 Ghanaian afebrile schoolchildren,[28] in 0.9% of the 447 febrile people in northern Angola,[29] and in 4.2% of 1,155 afebrile people in Malawi.[30] Thus, triple infection with these malarial species would appear to be underestimated when microscopy alone is used. Very few mono-infections with *P. malariae* and *P. ovale* were observed as they are most often associated with *P. falciparum*. Taken individually, the prevalence of *P. malariae* and *P. ovale* was the same between febrile and afebrile children. In addition, we found a high prevalence of *Plasmodium* spp. in afebrile children compared with that found in recent studies in cities in southern and southeastern Gabon.[19,20] Forest dominates the northeast of Gabon, whereas the savannah and steppes dominate the south and southeast. This may explain why the circulation of malaria and asymptomatic malaria is higher in Makokou (northeastern Gabon) compared with the areas previously studied.[21] Asymptomatic carriage could be an obstacle to the eradication of malaria.[31,32]

Filariases due to *M. perstans* and *L. loa* were also detected. Thus, *M. perstans*, which is not currently associated with a specific clinical picture and is often asymptomatic, was only observed in febrile patients only, and the well-known pathogen, *L. loa*, was present in both febrile and afebrile patients. These data suggest that more attention should be paid to *M. perstans*. Bacteremia was less common than parasitic diseases but only affected febrile children. Recent surveys conducted in Franceville and in other rural and urban cities in south and southeast Gabon reported almost similar prevalence rates (6% and 4.7% of 333 and 870 febrile patients, respectively).[19,20] Although *H. influenzae* was the most frequently identified bacterial species, its prevalence remains low (1.6%). In Gabon between 1989 and 1993, *H. influenzae* was revealed in a cerebrospinal fluid culture for 34.6% of the 104 cases of meningitis recorded, with a 31.4% mortality rate.[33] This decline of *H. influenzae* may be related to the official launch of the Gabonese government's vaccination campaign in April 2010 to promote a pentavalent vaccine containing the Hib vaccine (*H. influenzae* type b).[34] In the "Report on the WHO Cooperation Strategy with Gabon 2016–2021," immunization coverage with the pentavalent vaccine reached 82% according to the Expanded Programme on Immunization (EPI) report in 2012, and then experienced a steady decline to 70% according to the EPI in 2014. The prevalence of *S. aureus* and *S. pneumoniae* was as low as previously reported in other parts of Gabon.[19,20] The prevalence of *R. felis* was lower than that reported by Mourembou et al. in 2016, where it ranged from 1.3% (of 77 patients) in urban areas to 39.7% (of 58 patients) in rural areas.[19] Other

fastidious bacteria, such as *C. burnetii*, *Leptospira* spp., *T. whipplei*, and *B. quintana* were not detected, although these bacteria had already been identified as a cause of fever in parts of sub-Saharan Africa, such as Senegal, Tanzania, and Uganda.[11,12,14–17,23]

Our study also highlights the phenomenon of self-medication at home with anti-infective drugs and the gap between anti-infective treatments prescribed during hospitalization and retrospective molecular diagnosis. Home management of malaria (HMM) using ACT had been advocated to increase access to effective antimalarial drugs for high-risk groups living in underserved areas in sub-Saharan Africa. In Gabon, HMM has not been formally adopted by health authorities. However, self-medication at home is practiced by people living in rural areas, as shown by our study. Access to health care is difficult in remote areas of Gabon, as is the case in Nigeria and Ghana, where this malaria control strategy has been successfully implemented but under the control of health workers.[35,36] Indeed, in the absence of control, self-medication can be dangerous because of the inappropriate use of drugs, such as poor compliance or lack of adequate doses. In Nigeria, a study on the impact of HMM showed that there was no significant difference in parasitemia between children who received antimalarial treatment before the consultation and those who did not receive it. However, in the self-medication group, the prevalence of severe malaria was higher, with a mortality rate of 62/1,000 only in this group.[37] In addition, this practice could lead to the emergence of resistance to ACT, as reported in Asia.[38] Self-medication with antibiotics has also been observed. Such a practice could skew the results of etiological fever research, hinder the effectiveness of the antibiotics used, and lead to a waste of limited resources.[39] The gap between hospital-prescribed treatment and retrospective molecular diagnosis is remarkable. Almost a quarter of children with malaria were not given antimalarial treatment; conversely, 37.4% without malaria received antimalarial treatment. These data corroborate previous reports suggesting that 80% of children who are at high risk of developing severe malaria around the world are undertreated for malaria, whereas children without malaria are treated with antimalarial drugs.[6,40,41] Overall, antibiotics appear to be overused because although we did not carry out blood cultures, we searched for the main bacteria reported in the literature as being responsible for bacteremia in sub-Saharan Africa, particularly in Gabon. Previous studies predicted that the systemic use of RDT for the diagnosis of malaria, as recommended by WHO, could lead to the overprescription of antibiotics (in RDT negative patients) because of lack of knowledge about the causes of non-malarial fever.[42,43] Although the indiscriminate use of antibiotics has been used as a rescue strategy in areas where bacterial culture facilities are lacking, the misuse of antibiotics leads to selective pressure of antibiotic resistance.[39] None of the individuals who were infected by *S. pneumoniae* received antibiotics. Pneumococcal disease is a common cause of death in children worldwide,[35,36] although it is treatable and preventable by vaccination.[44,45] However, no deaths were recorded in our patients. This result shows that antibiotics are prescribed empirically, probably because of the shortage of blood culture facilities.

Our study presents some limitations, including lack of demographic data about afebrile children, small size of the afebrile children group, lack of histories of epidemiological exposure from the children, and exclusive use of PCR assays targeting specific microorganisms. Indeed, because of the lack of clinical diagnostic capacity in the study area, which is typical of many parts of Africa, we privileged the use of PCR assays, as we have done previously in Senegal and other areas in Gabon.[14,19,20,46] Thus, we only perform PCR assays targeting a selection of relevant pathogens. Polymerase chain reaction assays have a very low detection threshold and also allow the detection of DNA from a microorganism even if the patient has recently taken an anti-infective treatment. This very low threshold of detection by molecular biology can complicate the interpretation of the role of *Plasmodium* spp. in febrile episodes compared with light microscopy or RDTs. However, there are also drawbacks to these techniques, such as the requirement to have well-trained and experienced staff for light microscopy, the inability to quantify parasites, occasional false-positive results because of histidine-rich protein 2 (HRP2) antigenemia persisting in the absence of viable parasites, and false-negative results in case of the presence of high level of anti-HRP2 antibodies in humans or mutations in the HRP2 gene for HRP2-based malaria RDTs.[47,48] The impact of febrile bacterial infections was likely to be underestimated by the use of molecular analyses from blood samples targeting only a range of bacteria and which, for example, did not enable the diagnosis of bacterial otitis or urinary tract infections, which are considered to be common causes of fever, especially in young children.

Overall, at least one microorganism was observed in 74% of cases of fever. Expansion of the repertory study to other microorganisms such as respiratory or gastrointestinal viruses should provide a better understanding of undocumented fever cases. Malaria remains a major health problem in Makokou. Malaria control measures should be reassessed and rehabilitated in this region.

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