Travel Medicine and Infectious Disease 43 (2021) 102105

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Assessment of the burden of malaria and bacteraemia by retrospective molecular diagnosis in febrile illnesses and first-line anti-infectives in Côte d'Ivoire

Bilé Cyrille Hervé Ehounoud [a,b,c,d,1,†], Celia Scherelle Boumbanda Koyo [a,b,e,f,1], Landry Doua Bongue [g], Sébastien Cortaredona [a,b], Adèle N'Douba Kakou [h], Djanwai Berenger Konan [g], Yao Kouassi Patrick [c], Nadia Amanzougaghene [b,i], Jean-David N'Guessan [c], Bernard Davoust [b,i], Didier Raoult [b,i], Oleg Mediannikov [b,i], Florence Fenollar [a,b,*]

[a] Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France
[b] IHU-Méditerranée Infection, Marseille, France
[c] Félix Houphouët Boigny Université, UFR Biosciences, the Republic of Côte d'Ivoire
[d] Institut Pierre Richet, Institut National de Santé Publique, Bouaké, the Republic of Côte d'Ivoire
[e] Unité D'Evolution, Epidémiologie et Résistances Parasitaires (UNEEREP), Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon
[f] Ecole Doctorale Régionale en Infectiologie Tropicale D'Afrique Centrale, Franceville, Gabon
[g] Centre Médico Social Wale, BP 1336, Yamoussoukro, the Republic of Côte d'Ivoire
[h] Faculté de Médecine, Pharmacie et Odonto-Stomatologie, Université Félix Houphouët Boigny, the Republic of Côte d'Ivoire
[i] Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France

ARTICLE INFO

Keywords:
Fever
Bacteraemia
Malaria
Côte d'Ivoire
Salmonella enterica
Streptococcus pneumoniae

ABSTRACT

Background: The aetiologies of fever are poorly understood in sub-Saharan Africa. We aimed to assess the burden of malaria and bacteria in Côte d'Ivoire.
Methods: Blood samples from 438 febrile and 346 afebrile people were screened using molecular tools.
Results: Plasmodium falciparum was the most common microorganism associated with fever (46.8% in febrile, 23.4% in afebrile people; $p < 0.001$). Bacteraemia was detected in 21.7% of febrile people and 12.7% of afebrile people ($p = 0.001$). *Streptococcus pneumoniae* was the main cause of bacteraemia (7.1% of febrile and 0.6% of afebrile individuals; $p < 0.001$). Non-typhoidal *Salmonella* spp. was detected in 4.5% of febrile people and 1.2% of afebrile individuals ($p < 0.001$). *Salmonella enterica* Typhi and *S. enterica* Paratyphi were only detected in febrile subjects (1.4% and 2.1%), as well as *Tropheryma whipplei* (0.9%), *Streptococcus pyogenes* (0.7%), and *Plasmodium ovale* (4.6%). The prevalence in febrile and afebrile people was similar for *Staphylococcus aureus* (3.6–4.9%), *Rickettsia felis* (5.5–6.4%), *Mansonella perstans* (3.0–3.2%), and *Plasmodium malariae* (1.6–2.3%). Comorbidities were higher in febrile than in afebrile subjects (10.3% versus 5.5%; $p = 0.01$); 82% involving *P. falciparum*. All patients co-infected with *P. falciparum* and *S. pneumoniae* were febrile whereas 30% of those infected by *P. falciparum* alone were not ($p = 0.02$). Among febrile participants, 30.4% with malaria and 54.7% with bacteraemia had received neither antimalarial nor antibiotic therapy.
Conclusion: Identification of etiologies of acute febrile diseases in sub-Saharan Africa proposes keys to successful treatment and prevention of infectious diseases. Vaccination campaigns may decrease the morbidity of mono- and co-infections by preventable microorganisms.

* Corresponding author. Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France.
E-mail address: florence.fenollar@univ-amu.fr (F. Fenollar).
[1] Equally contributed.
[†] Doctor Bilé Cyrille Hervé EHOUNOUD passed away in August 2020.

https://doi.org/10.1016/j.tmaid.2021.102105
Received 6 May 2020; Received in revised form 21 April 2021; Accepted 4 June 2021
Available online 17 June 2021

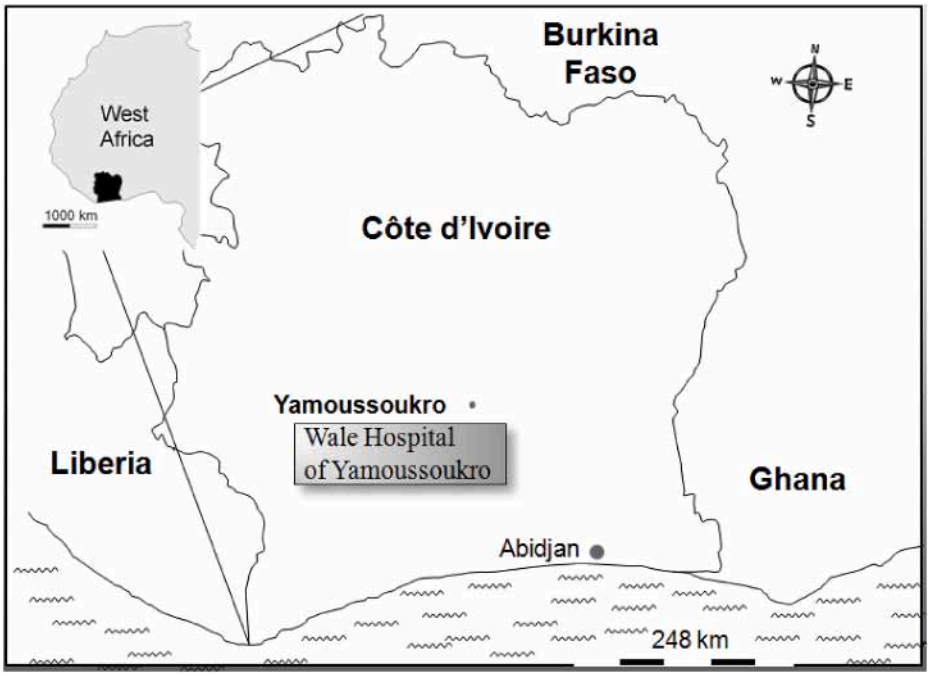

Fig. 1. Map of the study site in Côte d'Ivoire.

1. Introduction

Despite a substantial decrease in malaria burden in several areas of sub-Saharan Africa [1–3], there is a lack of knowledge around non-malaria febrile illnesses in many countries in this area. In addition, the overlapping of clinical signs between malaria and other febrile illnesses makes differential diagnosis of fever difficult in resource-limited settings [4,5]. The establishment of the local epidemiology of fever aetiologies is critical in order to optimise the management of febrile patients and to ensure a better use of available drugs.

The burden of bacterial aetiologies is poorly understood. Traditional diagnostic tools, such as blood cultures, are available almost exclusively in large cities. Furthermore, blood cultures do not allow the isolation of intracellular or highly fastidious bacteria. Only common bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Salmonella* spp. are regarded as the main bacterial aetiologies of fever [6,7]. Recently, molecular strategies for the characterisation of bacterial bloodstream infections enabled to highlight the implication of fastidious bacteria such as *Rickettsia felis* and *Tropheryma whipplei* in Senegal and Gabon [8, 9].

In Côte d'Ivoire between 1996 and 1998, studies investigating bacteraemia among febrile HIV-infected patients identified non-typhoidal *Salmonella enterica* (15), *S. pneumoniae* (13), *Escherichia coli* (6), *Shigella* spp. (2), *S. aureus* (2), *Proteus mirabilis* (1), and *Salmonella enterica* Typhi (1) as bacterial aetiologies [10]. More recently, between 2012 and 2014, after a long period when culture equipment was unavailable, a study on the morbidity related to bacterial blood infections was conducted in Bouaké (central Côte d'Ivoire) with *Klebsiella pneumoniae* and *S. enterica* being involved in 94% of bloodstream infections followed by *S. aureus* [11].

Although there is evidence of the circulation of zoonotic bacteria such as *C. burnetii*, *Rickettsia* spp., *Borrelia* spp., and *Anaplasma* spp. in cattle, sheep, goats, and ticks in Côte d'Ivoire [12,13], their role in human febrile illness has not yet been thoroughly investigated.

Our study, therefore, aimed to determine the burden of malaria, Mansonellosis, common and fastidious bacteria in Yamoussoukro (Côte d'Ivoire) using specific molecular tools and to compare the accuracy of treatments prescribed in the hospital and the retrospective molecular diagnosis.

2. Material and methods

2.1. Ethics statement

The Côte d'Ivoire National Research Ethics Committee and the Wale Hospital of Yamoussoukro (agreement number 86 MSLS/CNER-dkn) approved the study protocol. Written informed consent was obtained from each adult subject and from the parents or legal guardians of children included in the study.

2.2. Study site

The study was carried out at the Wale hospital in Yamoussoukro in Côte d'Ivoire (West Africa). Yamoussoukro is the political and administrative capital of the country and is located 248 km north of Abidjan, the economic capital (Fig. 1). In the district, the vegetation is dominated by wet savanna with varying small trees. This area is characterised by its mean temperature of 26 °C and the rainy season peaks between March–July, and September–October. The district of Yamoussoukro has a total of 362,000 inhabitants, of whom 199,100 (55%) live in urban areas.

2.3. Study population and sample collection

This is a cross-sectional epidemiological study. Between September 2014 and April 2015, 438 people consulting at the hospital for fever (axillary temperature $\geq$ 37.5 °C) were recruited as well as comparative group of 346 asymptomatic people recruited at the same time. The afebrile comparative group consisted of asymptomatic volunteers, parents and children accompanying people seeking medical care, and who did not have episodes of fever in the previous month.

Three drops of blood from each participant (about 200 μL) were collected using the finger prick method and collected in an Eppendorf tube containing 20 μL of citrate. On site, DNA was extracted with QIAamp DNA kit (Qiagen, Hilden, Germany) in line with the manufacturer's protocol without elution. The columns of DNA were stored in the refrigerator before being transferred to the Institut Hospitalo-Universitaire Mediterranée Infection (Marseille, France), where DNA elution was performed with 200 μL of buffer AE (Acetone, EDTA). DNA

Table 1
Demographic and clinical characteristics of the population studied.

	438 febrile patients	346 afebrile people
	Number (%)	
Male sex	176 (40.3)	201 (58.1)
Age group (years)		
<5	222 (50.8)	13 (3.8)
5–20	119 (27.2)	314 (90.7)
>20	96 (22.0)	19 (5.5)
Complaints		
Non-specific		
Headache	243 (55.5)	35 (10.1)
Asthenia	202 (46.1)	7 (2.0)
Rhinorrhea	73 (16.7)	0
Gastrointestinal symptoms	**222 (50.7)**	**0**
Vomiting	136 (31.1)	0
Nausea	130 (29.7)	0
Abdominal pain	62 (14.2)	0
Diarrhea	27 (6.2)	0
Respiratory symptoms	**190 (43.4)**	**0**
Cough	162 (37.0)	0
Expectoration	7 (1.6)	0
Dyspnea	23 (5.3)	0

Missing values for age (n = 1) and sex (n = 1) in febrile individuals.

was stored at +4 °C until used for PCR analysis.

2.4. Assessment of DNA quality and molecular analysis

DNA quality was assessed using quantitative real-time PCR (qPCR) targeting the human β-actin gene, as previously reported [14]. After verifying the quality of DNA extracts, they were screened for the presence of *Plasmodium* spp., *S. pneumoniae*, *Staphylococcus aureus*, *Salmonella* spp., *S. pyogenes*, *Rickettsia* spp., *Borrelia* spp., *T. whipplei, C. burnetii, Bartonella* spp., *Leptospira* spp., *Anaplasma* spp., and *Mansonella* spp. using qPCR. The primers and probes used for each of the targeted microorganisms are presented in Table S1.

The final reaction volume (20 μL) of qPCR contained 10 μL of Master mix No ROX (Eurogentec, Liege, Belgium), 3.5 μL of distilled water (DNAase/RNAase free), 2.5 μM of probe, 20 μM of each primer, and 5 μL DNA. All reactions were carried using a CFX 96 (Bio-Rad, Marnes-la-Coquette, France) according to following settings: DNA denaturation steps at 50 °C for 2 min and 95 °C for 5 min followed by 40 cycles of 1 s at 95 °C, 35 s at 60 °C, and an extension step for 30 s at 45 °C. In each reaction, two positive controls (DNA of targeted microorganism) and two negative controls (mix alone) were used to validate each PCR assay.

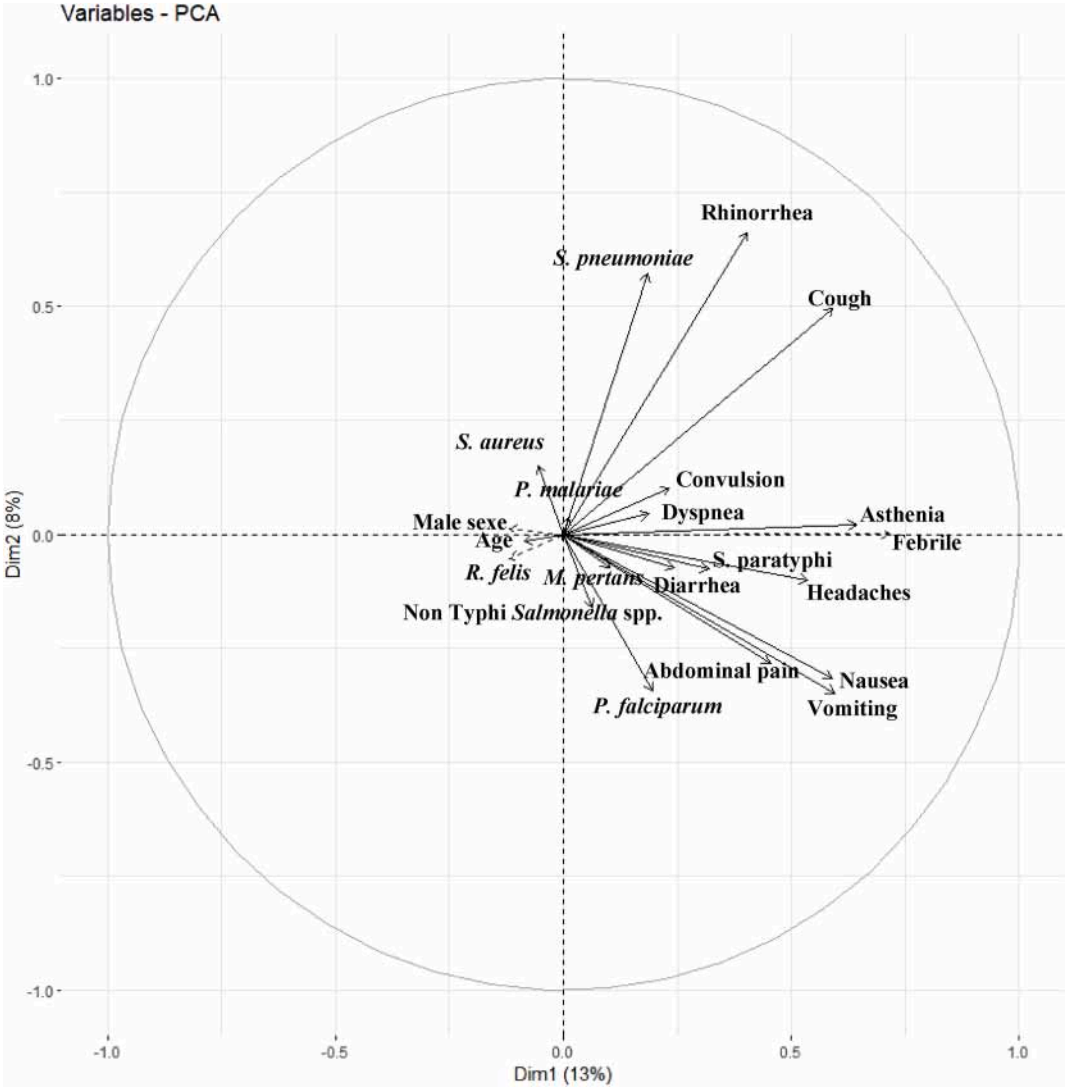

Fig. 2. Principal Component Analysis (PCA) of clinical and biological outcomes, demographic characteristics of 348 febrile patients and 346 afebrile people.

Table 2
Prevalence of microorganisms detected in 438 febrile patients and 346 afebrile people (A) and their prevalence by geographic origin in febrile patients (B).

A.	438 febrile patients	346 afebrile people	
Microorganisms	**Number of positive (%)**		***p.* value***
Bacteria			
S. pneumoniae	**31 (7.1)**	**2 (0.6)**	**< 0.001**
R. felis	24 (5.5)	22 (6.4)	0.603
Non-typhoidal *Salmonella enterica*	**18 (4.5)**	**4 (1.2)**	**< 0.001**
S. aureus	16 (3.6)	17 (4.9)	0.383
***S. enterica* Paratyphi**	**9 (2.1)**	**0**	**0.005**
***S. enterica* Typhi**	**6 (1.4)**	**0**	**0.030**
T. whipplei	4 (0.9)	0	0.134
S. pyogenes	3 (0.7)	0	0.259
Parasites			
***Plasmodium* spp.**	**217 (49.5)**	**85 (24.6)**	**< 0.001**
P. falciparum	**205 (46.8)**	**81 (23.4)**	**< 0.001**
P. ovale	**20 (4.6)**	**0**	**< 0.001**
P. malariae	7 (1.6)	8 (2.3)	0.468
Mansonella perstans	13 (3)	11 (3.2)	0.865

B.	322 febrile urban inhabitants	116 febrile rural inhabitants	
Microorganisms	**Number of positive (%)**		***p.* value**
Bacteria			
S. pneumoniae	20 (6.2)	11 (9.5)	0.239
R. felis	**12 (3.7)**	**12 (10.3)**	**0.007**
Non-typhoidal *Salmonella enterica*	12 (3.7)	6 (5.2)	0.501
S. aureus	12 (3.7)	4 (3.5)	1.000
S. enterica Paratyphi	7 (2.2)	2 (1.7)	0.770
***S. enterica* Typhi**	**2 (0.6)**	**4 (3.5)**	**0.045**
T. whipplei	4 (1.2)	0	0.500
S. pyogenes	2 (0.6)	1 (0.9)	1.000
Parasites			
***Plasmodium* spp.**	**145 (45.0)**	**72 (62.1)**	**< 0.001**
P. falciparum	**135 (41.9)**	**70 (60.3)**	**0.007**
P. ovale	15 (4.7)	5 (4.3)	0.878
P. malariae	3 (0.9)	4 (3.4)	0.064
M. perstans	**6 (1.0)**	**7 (6.0)**	**0.023**

No samples were positive for *C. burnetii*, *Borrelia* spp., *Bartonella* spp., and *Leptospira* spp.
* Significant p. value (<0.05) are in bold (Chi-square or Fisher's exact tests, 2-sided p. values).

2.5. Statistical analysis

Statistical analyses were performed using SAS university edition software (version 9.4, SAS Institute Inc., Cary, NC, USA) and R software (version 3.6.2). Chi-square's and Fischer's tests were used to compare frequencies between febrile and afebrile subjects. For statistical significance, the two-sided *p. values* were set at 0.005. Principal Component Analysis (PCA) was performed to assess the relationship between relevant pathogens, demographic characteristics, fever, and other clinical signs.

3. Results

The average age of study participants was 20 ± 12-year-old (range 3 weeks–71 years old). Three hundred and forty-four febrile patients were urban residents and 94 came from rural areas.

The quality of the DNA extracted from all blood samples was satisfactory, given that the cycle threshold values (Ct) of qPCR targeting human β-actin gene ranged from 19 to 26. The demographic and clinical characteristics of the population studied are summarised in Table 1. Principal Component Analysis showed that *P. falciparum* and *S. pneumoniae* were strongly correlated with febrile status, asthenia, cough, rhinorrhea, headaches, vomiting, and nausea (Fig. 2). Otherwise, non-typhoidal *Salmonella*, *S. enterica* Paratyphi, diarrhea, and abdominal pain were strongly correlated.

3.1. Parasitaemia

3.1.1. Malaria

Plasmodium parasites were the more frequently detected microorganisms with an overall prevalence of 38.5% (302/784). They were also significantly higher in febrile (49.5%, 217/438) than in afebrile subjects (24.6%, 85/346; $p < 0.001$) (Table 2). Among febrile patients, *P. falciparum* was more frequently detected in rural (62.1%, 72/116) than in urban subjects (45%, 145/322; $p < 0.001$), while no statistical differences were observed between rural and urban areas for *P. ovale* and *P. malariae* (Table 2). *P. falciparum* represented 94.7% (286/302) of samples which were positive for *Plasmodium* species. Its prevalence was significantly higher in febrile (46.8%, 205/438) than in afebrile subjects (23.4%, 81/346; $p < 0.0001$). *P. ovale* was only identified in febrile patients (4.6%, 20/438). *P. malariae* was less common in febrile patients (1.6%, 7/438) than in afebrile subjects (2.3%, 8/346; $p = 0.468$). In febrile people, the prevalence of malaria due to *P. falciparum* decreased with age ($p < 0.001$) (Fig. 3, Table 3). For *P. malariae* and *P. ovale*, no statistical differences were observed between age groups (Table 3).

3.1.2. Mansonella perstans

Filariasis due to *M. perstans* was detected in 3.1% (20/784) of subjects, including 3.0% (13/438) of febrile and 3.2% (11/346; $p = 0.865$) of afebrile individuals (Fig. 3). In febrile patients, the prevalence of *M. perstans* was significantly higher among those living in rural areas (6%, 7/116) as opposed to urban areas (1%, 6/322; $p = 0.023$). The prevalence of *M. perstans* also increased significantly with age in febrile subjects (Table 3).

3.2. Bacteraemia

Overall, 21.7% of febrile people (95/438) had bacteraemia versus 12.7% of afebrile people (44/346; $p = 0.001$). *S. pneumoniae* was the second most frequently detected microorganism, detected in 4.2% (33/784) of positive subjects. The prevalence of *S. pneumoniae* was significantly higher in febrile people (7.1%, 31/438) than in afebrile people (0.6%, 2/346; $p < 0.001$). No significant difference was observed between febrile people living in urban or rural areas (Table 2). In febrile people, *S. pneumoniae* was more frequently detected in children under five years old (15.1%, 19/126), followed by the 5–20 year old age group (2.4%, 3/123) and finally those aged over the age of 20 (4.8%, 9/189; $p = 0.0003$) (Table 3). The two afebrile people who were positive for *S. pneumoniae* were 22 and 23 years old.

Salmonella spp. was more frequently identified in febrile subjects (7.5%, 33/438) than in afebrile subjects (1.2%, 4/346, $p < 0.001$). Non-typhoidal *Salmonella* was identified only in afebrile people (1.2%, 4/346) but was significantly associated with fever ($p < 0.001$). Indeed, non-typhoidal *Salmonella* was the most frequently detected species in febrile subjects (4.5%, 18/438), followed by *Salmonella enterica* Paratyphi (2.1%, 9/438), and *Salmonella enterica* Typhi (1.4%, 6/438). No significant difference was observed between febrile people living in rural and urban areas for non-typhoidal *Salmonella* and *S. enterica* Paratyphi (Table 2) while *S. enterica* Typhi was significantly higher in rural areas (3.5%, 4/116) compared to urban areas (0.6%, 2/322; $p = 0.045$). For non-typhoidal *Salmonella* and *S. enterica* Paratyphi, no statistical difference was observed between age groups, whereas *S. enterica* Typhi was only observed in children under the age of five (4.8%, 6/126; $p = 0.001$).

S. aureus was detected in 4.2% (33/784) of subjects, more precisely in 3.6% (16/438) of febrile people and 4.9% of afebrile people (17/346, $p = 0.383$). No statistical difference was observed according to location

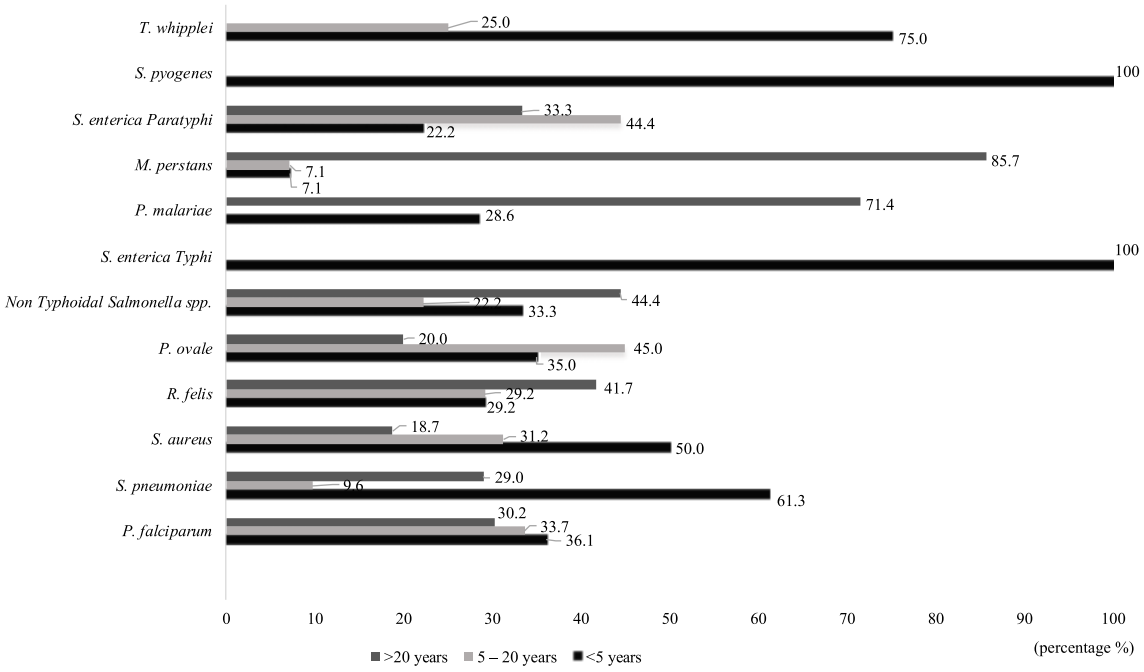

Fig. 3. Prevalence of microorganisms detected among the 438 febrile patients according to age.

Table 3
Prevalence of microorganisms according to age groups in febrile people.

	Age groups (Years)			
	< 5	5–20	> 20	
	Number of febrile people			
	126	123	189	
Microorganisms	**Number of positive (%)**			***p.* value***
Bacteria				
S. pneumoniae	**19 (15.1)**	**3 (2.4)**	**9 (4.8)**	**0.003**
R. felis	7 (5.6)	7 (5.7)	10 (5.3)	0.999
Non-typhoidal *Salmonella enterica*	6 (4.8)	4 (3.3)	8 (4.2)	0.869
S. aureus	8 (6.4)	5 (4.1)	3 (1.6)	0.075
Salmonella enterica Paratyphi	2 (1.6)	4 (3.3)	3 (1.6)	0.632
***Salmonella enterica* Typhi**	**6 (4.8)**	**0**	**0**	**0.001**
T. whipplei	3 (2.4)	1 (0.8)	0	0.064
S. pyogenes	**3 (2.4)**	**0**	**0**	**0.045**
Parasites				
P. falciparum	**74 (58.7)**	**69 (56.1)**	**62 (32.8)**	**< 0.001**
P. ovale	7 (5.6)	9 (7.3)	4 (2.1)	0.064
P. malariae	2 (1.6)	0	5 (2.7)	0.215
M. perstans	**1 (0.8)**	**1 (0.6)**	**12 (6.4)**	**0.001**

* Significant *p. value* (<0.05) are in bold (Chi-square or Fisher's exact tests, 2-sided *p. value*).

or age. *S. pyogenes* was only detected in three febrile children under the age of five (Table 3).

The prevalence of *R. felis* was 5.9% (46/784) among all individuals, of whom 5.5% were febrile (24/438) and 6.4% were afebrile (24/364, $p = 0.603$). Its prevalence in febrile patients was significantly higher in rural areas (10.3%, 12/322) than in urban areas (3.7%, 12/322; $p = 0.007$). *T. whipplei* was only identified in four febrile children under the age of eight, all of whom lived in an urban area.

3.3. Co-infections

Co-infections were significantly higher in febrile patients (10.3%, 45/438) than in afebrile people (5.5%, 19/346; $p = 0.010$). Most of the co-infections involved *Plasmodium falciparum* (82.8%, 53/64). In addition, of the 286 patients who tested positive for *P. falciparum*, the probability of being febrile was significantly higher in patients co-infected with *S. pneumoniae* (12/12, 100%) than in mono-infected patients (193/274, 70.0%; $p = 0.020$) (Table S2). Details of co-infections are summarised in Table 4.

Table 4
Prevalence of co-infections among 438 febrile and 346 afebrile people.

	438 febrile patients	346 afebrile people	
	Number of positive (%)		***p.* value**
Co-infections	**45 (10.3%)**	**19 (5.5%)**	**0.01**
Triple infections	**8 (1.8%)**	**2 (0.6%)**	**0.100**
P. falciparum/M. perstans/Non-typhoidal *Salmonella*	0	1 (0.3)	0.441
P. falciparum/P. malariae/S. aureus	0	1 (0.3)	0.441
P. falciparum/P. malariae/S. pneumoniae	1 (0.2)	0	0.999
P. falciparum/P. ovale/R. felis	1 (0.2)	0	0.999
P. falciparum/P. ovale/S. aureus	2 (0.5)	0	0.506
P. falciparum/R. felis/S. aureus	1 (0.2)	0	1.000
P. falciparum/S. aureus/S. pneumoniae	2 (0.5)	0	0.506
P. ovale/S. aureus/S. enterica Typhi	1 (0.2)	0	0.999
Double infections	**37 (8.5%)**	**17 (4.9%)**	**0.05**
P. falciparum/M. perstans	2 (0.5)	3 (0.9)	0.659
P. falciparum/R. felis	8 (1.8)	6 (1.7)	0.922
P. falciparum/S. aureus	10 (2.3)	3 (0.9)	0.162
P. falciparum/Non-typhoidal *Salmonella*	10 (2.3)	2 (0.6)	0.077
Non-typhoidal *Salmonella/M. perstans*	2 (0.5)	1 (0.3)	0.999
Non-typhoidal *Salmonella/T. whipplei*	2 (0.5)	0	0.999
R. felis/S. aureus	1 (0.2)	1 (0.3)	0.999
R. felis/Non-typhoidal *Salmonella*	1 (0.2)	0	0.999
R. felis/S. enterica Typhi	1 (0.2)	0	0.999
R. felis/M. perstans	0	1 (0.3)	0.441

Table 5
Retrospective molecular diagnosis of bacteremia and respective antibiotic treatment prescribed at admission to hospital in febrile patients.

Retrospective molecular diagnosis	Antibiotic treatment at admission	Number of patients
S. pneumoniae	Amoxicillin	8
	Amoxicillin and clavulanic acid	1
	Amoxicillin/Metronidazole	1
	Cefadroxil	2
	Rifamycin/Enrofloxacin/ Cefpodoxime	1
	Cefadroxil/Ethambutol	1
R. felis	Amoxicillin	2
	Cefadroxil	1
	Neomycin/Bacitracin	1
	Metronidazole	1
	Penicillin	1
S. enterica Typhi	Amoxicillin and clavulanic acid	3
	Cefadroxil	2
	Nifuroxazide	1
	Amoxicillin and clavulanic acid	1
Non-typhoidal *Salmonella* spp.	Amoxicillin	4
	Azythromycin	1
	Amoxicillin and clavulanic acid	3
S. enterica Paratyhi/*S. aureus*	Amoxicillin	1
	Amoxicillin and clavulanic acid	1
S. aureus/S. pneumoniae	Amoxicillin	1
	Cefadroxil	1
S. enterica Paratyhi	Amoxicillin	1
S. aureus	Flucloxacillin	1
R. felis/S. enterica Typhi	Cefadroxil	1
T. whipplei/non-typhoidal *Salmonella* spp.	Amoxicillin and clavulanic acid	1

3.4. Treatment prescribed at hospital and retrospective molecular diagnostic

Treatments prescribed at the hospital were compared to retrospective molecular diagnosis. Of the 95 patients with bacteraemia, 52 did not received antibiotic treatment. Of them, 15 were infected with *R. felis*, 10 with *S. pneumoniae*, 7 with non-typhoidal *Salmonella*, 6 with *S. aureus*, 3 with *S. enterica* Paratyphi, one with *S. enterica* Typhi, and one with *S. pyogenes*. Nine of the 52 patients who did not receive an antibiotic prescription had a mixed bacterial infection. One of them were co-infected with *R. felis* and *S. aureus*, one with *R. felis* and non-typhoidal *Salmonella*, 3 three with *S. aureus* and *S. pneumoniae*, one with *S. pyogenes* and *S. aureus*, one with *S. pyogenes* and *S. enterica* Typhi, and 2 with *T. whipplei* and *S. pneumoniae*. Forty-three of the 95 patients with bacteraemia received antibiotics. Retrospective molecular diagnosis of bacteraemia and antibiotics prescribed when febrile patients were admitted to hospital are summarised in Table 5. Conversely, 133 of 343 patients without bacteraemia linked with screened bacteria received antibiotics (39 combinations of amoxicillin and clavulanic acid, 35 amoxicillin, one combination of amoxicillin, clavulanic acid and ampicillin, 11 cefadroxil, 12 cefpodoxime, 3 azithromycin, 16 ciprofloxacin, one enrofloxacin, 2 erythromycin, one flucloxacillin, one imidazole, and 11 metronidazole).

Overall, 66 of the 217 febrile patients (30.4%) who were positive for *Plasmodium* spp. did not received antimalarial drugs (Fig. 4). Of these, 56 were infected with *P. falciparum* alone, 2 two with *P. falciparum* and *P. malariae,* 3 with *P. malariae* alone, and 5 with *P. ovale* alone. Conversely, 116 (52.5%) of patients without malaria received antimalarial drugs (Fig. 4). Of these, 109 received artemether-lumefantrine, one received artesunate-amodiaquine, 3 quinine, 2 sulfadoxine-pyrimethamine, and one received piperaquine.

4. Discussion

Malaria is the main aetiology in patients consulting for fever at the Wale Hospital in Yamoussoukro, with *P. falciparum* being the most frequently identified species. Previous studies in Côte d'Ivoire were performed using the thick smear procedure, which is known to be less sensitive than molecular tools. In 2008, the prevalence of malaria at the Abobo Hospital in Abidjan was estimated at 29.5% among 902 febrile people and 13.5% among 681 afebrile people [15]. However, in the town of Tabou in 2010 and 2011 the prevalence of malaria was estimated at between 55.1% of 147 and 67.1% of 158 febrile people, and between 45% of 948 to 56.8% of 1029 afebrile people, respectively) [16]. In addition to the difference of sensitivity of each tool, geographic differences could explain the disparity of malaria prevalence, as Yamoussoukro alternates between forest and savannah [17] while Abobo is in a plateau area and Tabou is predominantly equatorial forest. Overall, a high rate of asymptomatic malaria was observed, as has been reported in other countries with high malaria transmission [18,19]. This high number of asymptomatic infections poses a challenge to malaria elimination efforts [20]. Finally, malaria was more prevalent among younger people and in rural areas, as previously reported [1,16,21–24]. The prevalence of *M. perstans* was similar in febrile and afebrile subjects. Indeed, *M. perstans* is quite often asymptomatic in populations in endemic areas [25]. Conversely, *M. perstans* may be responsible for invasive infections for people from non-endemic areas coming to endemic areas in Africa or America [25,26]. As already observed, *M. perstans* is significantly more common among adult and rural populations [27,28].

Bacteraemia was found in 21.7% of patients, with *S. pneumoniae* being the leading aetiological agent and strongly associated with fever. A first study on the morbidity of bloodstream infections, conducted using blood cultures between 2012 and 2014 in Côte d'Ivoire, found similar bacteraemia rates (22.5%, 293) but did not find *S. pneumoniae* [11]. Nevertheless, based on a previous study carried out in Côte d'Ivoire between 1999 and 2013, on aetiologies of meningitis among children, *S. pneumoniae* was responsible for 44% of meningitis cases [29]. Although *S. pneumoniae* is the main cause of febrile bacteraemia, no fatal cases were observed in our study.

S. enterica Typhi and Paratyphi were only observed in febrile people while non-typhoidal *Salmonella,* although significantly more prevalent in febrile people, was also detected in afebrile people. In addition, just over half of the people infected with non-typhoid *Salmonella* were co-infected with *P. falciparum.* A positive correlation between *Salmonella* bacteraemia and malaria has been reported for a long time [30].

Although *S. aureus* has already been reported as a main cause of community acquired bloodstream infections in Africa [31], its prevalence was similar in febrile and afebrile subjects. Asymptomatic carriage of *S. aureus* is common in humans [32,33]. Thus, contamination by skin microbiota during blood sampling should be mentioned. However, asymptomatic *S. aureus* bacteraemia, by analogy with asymptomatic malaria, cannot be fully excluded. Finally, *S. pyogenes* was only very rarely observed and only in febrile children.

R. felis was already detected in one *Ctenocephalides canis* collected from a dog in Bouaké, Côte d'Ivoire [34]. However, to the best of our knowledge, this is the first report of *R. felis* bacteraemia in this country. *R. felis* bacteraemia has already been reported in west Africa (Senegal), central Africa (Gabon), and east Africa (Kenya) mainly in febrile people but also in afebrile people [8,35,36]. In Gabon, in the rural area of Fougamou, its prevalence reached 39.7% (23/58) in febrile children, compared to 5.0% (1/20) in afebrile children in the same area and 1.3% (1/77) in febrile children in the city of Franceville [8]. In our study, no difference was observed between febrile and afebrile people, but the prevalence of *R. felis* bacteraemia was significantly higher in rural areas. Finally, *R. felis* was also detected in Senegal in skin swabs from eschars in acute febrile patients and healthy villagers, but not in those of French urban residents [35,37].

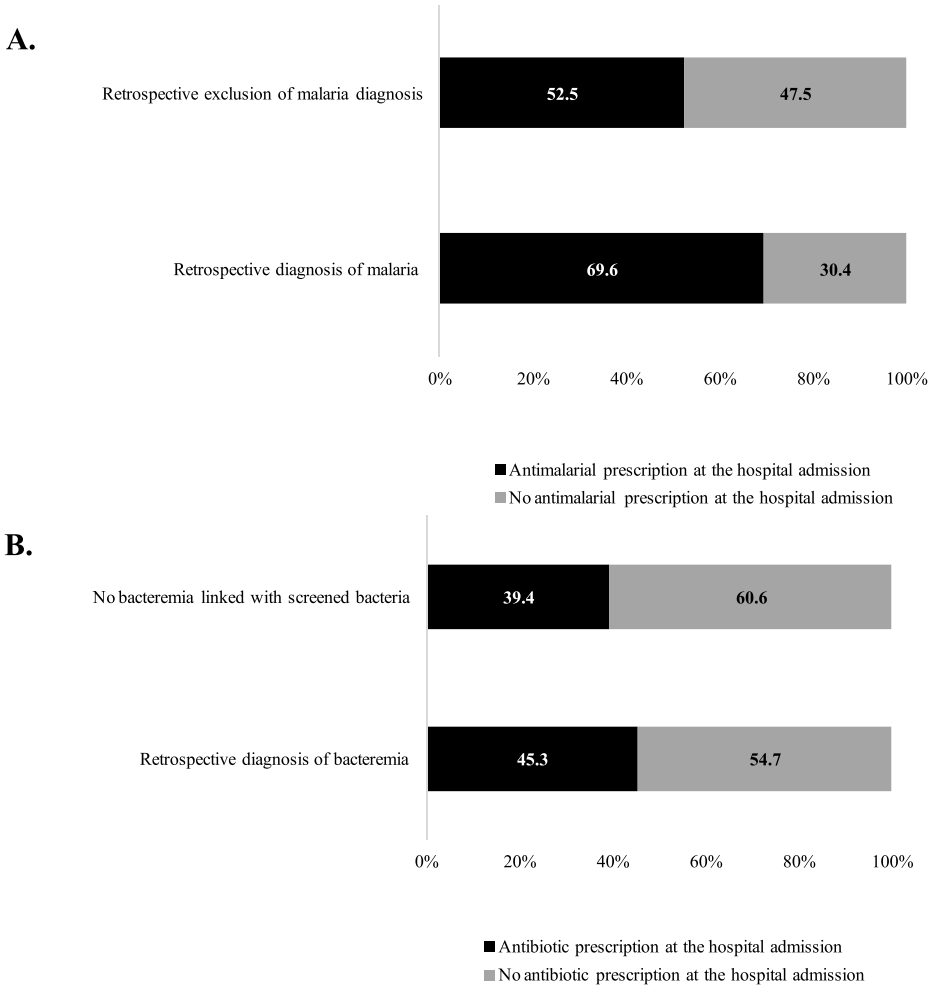

Fig. 4. Rate of antimalarial (A) and antibiotic (B) prescriptions upon admission to hospital compared to retrospective molecular diagnostic outcomes.

To the best of our knowledge, this is also the first report in Côte d'Ivoire of *T. whipplei,* the agent of Whipple's disease [38]. In rural Africa, *T. whipplei* has already been detected in faeces, mainly from young, asymptomatic and diarrhoeic children in Gabon, Ghana and Senegal [39–41]. Acute *T. whipplei* bacteraemia has also been reported in febrile people in rural areas of Senegal, including in young children presenting with a cough, but also as a cause of epidemic fever [42,43], and in a child from Lastoursville in rural Gabon [44,45].

Co-infections between malaria and bacterial bloodstream infections, mainly due to non-typhoid *Salmonella,* are commonly described in sub-Saharan Africa in febrile people and are associated with an increased risk of poor prognosis [46,47]. Surprisingly, for the first time we report a co-infection between *P. falciparum* and non-typhoid *Salmonella* in 3 afebrile children who had not experienced fever nor taken antibiotic or antimalarial treatment in the previous month. Of course, the molecular analysis used to screen for pathogens cannot provide any information on the viability of these microorganisms but the persistence of bacterial DNA in the blood has never been reported for acute infections, to the best of our knowledge. In addition, it should be noted that all patients co-infected with S. *pneumoniae* and *P. falciparum* were febrile. This suggests the further necessity to evaluate the efficacy of the vaccination against S. *pneumoniae* for the prevention febrile episodes linked to malaria.

The differences between the retrospective molecular diagnosis and the anti-infectives prescribed for hospital admission in febrile patients can easily be explained by the lack of reliable diagnostic tools available. A Ghanaian study found that 4.5% of the 247 children with a retrospectively confirmed diagnosis of malaria had not received antimalarial drugs, while up to 84.1% of the 446 children without malaria had received antimalarial drugs [48]. It is likely that the inappropriate use of anti-infectives is still common practice in sub-Saharan Africa. Indeed, the diversity of infectious aetiologies, the presence of co-infections, the prevalence of asymptomatic infections, the few or complete lack of diagnostic tools make the therapeutic choices available to physicians very difficult. Current WHO recommendations for the management of fever in sub-Saharan Africa are to identify signs of severity in patients with fever. If there are signs of seriousness, antimalarials and antibiotics are automatically prescribed before admission. After admission, rapid diagnostic testing for malaria and blood film are performed. If at least one test is positive, parenteral antimalarials should be given, and in other cases parenteral antibiotics should be administered before assessing for other causes of fever. If there is no sign of severity, a rapid malaria diagnostic test and a blood smear should be performed, and if at least one of them is positive, antimalarial treatments should be administered. In other cases, an antimalarial treatment should be prescribed and the patient must be monitored before trying to identify another cause of fever [49].

Improving the management of febrile illnesses in sub-Saharan Africa requires effort and resources that go beyond the deployment of rapid malaria tests. Indeed, it is also necessary to understand the repertoire of bacteria present in the ecosystems of the different regions in order to simultaneously answer the question that if it is not malaria, then what can it be, and which empirical antibiotic therapy would be the most appropriate. Although our study has some limitations (lack of traditional

blood cultures and lack of data about antibiotic susceptibility, differences in age/sex in febrile group of patients and comparative afebrile group, lack of data on RNA viruses as causative agents due to the logistic difficulties for the RNA conservation), it highlights the persistent presence of such pathogenic microorganisms as *S. pneumoniae* and *S. enterica* Typhi as well as possible association between co-infection *S. pneumoniae*/malaria with acute febrile episodes. Vaccines against these bacteria are available, so, vaccination campaigns in sub-Saharan Africa may decrease acute febrile morbidity associated with these preventable microorganisms.

Declaration of competing interest

The authors report no potential conflicts.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2021.102105.

Funding

The authors thank "l'Association Pour la Recherche en Infectiologie (APRI)" for funding some of the fieldwork. Our thanks also go to all those who agreed to take part in this study. We thank also the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the French National Research Agency for supporting this study, under the "Investissements d'avenir" programme, reference ANR-10-IAHU-03, the Région Provence Alpes Côte d'Azur and European funding FEDER IHUBIOTK.

References

[1] Cotter C, Sturrock HJW, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet 2013;382:900–11. https://doi.org/10.1016/S0140-6736(13)60310-4.

[2] Kouassi BL, de Souza DK, Goepogui A, Balde SM, Diakité L, Sagno A, et al. Low prevalence of *Plasmodium* and absence of malaria transmission in Conakry, Guinea: prospects for elimination. Malar J 2016;15:175. https://doi.org/10.1186/s12936-016-1230-9.

[3] WHO | world malaria report. Available: https://www.who.int/malaria/publications/world_malaria_report_2013/en/; 2013.

[4] Chipwaza B, Mhamphi GG, Ngatunga SD, Selemani M, Amuri M, Mugasa JP, et al. Prevalence of bacterial febrile illnesses in children in Kilosa district, Tanzania. PLoS Neglected Trop Dis 2015;9:e0003750. https://doi.org/10.1371/journal.pntd.0003750.

[5] Chipwaza B, Mugasa JP, Mayumana I, Amuri M, Makungu C, Gwakisa PS. Community knowledge and attitudes and health workers' practices regarding non-malaria febrile illnesses in eastern Tanzania. PLoS Neglected Trop Dis 2014;8:e2896. https://doi.org/10.1371/journal.pntd.0002896.

[6] Msemo OA, Mbwana J, Mahende C, Malabeja A, Gesase S, Crump JA, et al. Epidemiology and antimicrobial susceptibility of *Salmonella enterica* bloodstream isolates among febrile children in a rural district in Northeastern Tanzania: a Cross-sectional study. Clin Infect Dis 2019;68:S177–82. https://doi.org/10.1093/cid/ciy1126.

[7] Msefula CL, Olgemoeller F, Jambo N, Segula D, Van Tan T, Nyirenda TS, et al. Ascertaining the burden of invasive *Salmonella* disease in hospitalised febrile children aged under four years in Blantyre, Malawi. PLoS Neglected Trop Dis 2019;13:e0007539. https://doi.org/10.1371/journal.pntd.0007539.

[8] Mourembou G, Lekana-Douki JB, Mediannikov O, Nzondo SM, Kouna LC, Essone JCBB, et al. Possible role of *Rickettsia felis* in acute febrile illness among children in Gabon. Emerg Infect Dis 2015;21:1808–15. https://doi.org/10.3201/eid2110.141825.

[9] Sokhna C, Mediannikov O, Fenollar F, Bassene H, Diatta G, Tall A, et al. Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Neglected Trop Dis 2013;7:e1999. https://doi.org/10.1371/journal.pntd.0001999.

[10] Anglaret X, Dakoury-Dogbo N, Bonard D, Touré S, Combe P, Ouassa T, et al. Causes and empirical treatment of fever in HIV-infected adult outpatients, Abidjan, Côte d'Ivoire. AIDS 2002;16:909–18. https://doi.org/10.1097/00002030-200204120-00011.

[11] Akoua-Koffi C, Tia H, Plo JK, Monemo P, Cissé A, Yao C, et al. Epidemiology of community-onset bloodstream infections in Bouaké, central Côte d'Ivoire. New Microbes New Infect 2015;7:100–4. https://doi.org/10.1016/j.nmni.2015.06.009.

[12] Ehounoud CB, Yao KP, Dahmani M, Achi YL, Amanzougaghene N, Kacou N'Douba A, et al. Multiple pathogens including potential new species in tick vectors in Côte d'Ivoire. PLoS Neglected Trop Dis 2016;10:e0004367. https://doi.org/10.1371/journal.pntd.0004367.

[13] Kanouté YB, Gragnon BG, Schindler C, Bonfoh B, Schelling E. Epidemiology of brucellosis, Q Fever and Rift Valley Fever at the human and livestock interface in northern Côte d'Ivoire. Acta Trop 2017;165:66–75. https://doi.org/10.1016/j.actatropica.2016.02.012.

[14] Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez JF, et al. *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS Neglected Trop Dis 2010;4:e654. https://doi.org/10.1371/journal.pntd.0000654.

[15] Assoumou A, Adoubryn KD, Aboum KS, Kouadio-Yapo CG, Ouhon J. Symptomatic and asymptomatic *Plasmodium falciparum* infection in children from 6 months to 6 years old in the Abobo general hospital (Abidjan, Côte d'Ivoire). Bull Soc Pathol Exot 2008;101:50–3.

[16] Bassa FK, Ouattara M, Silué KD, Adiossan LG, Baikoro N, Koné S, et al. Epidemiology of malaria in the Taabo health and demographic surveillance system, south-central Côte d'Ivoire. Malar J 2016;15. https://doi.org/10.1186/s12936-015-1076-6.

[17] Konan E, Yaokokore-Beibro H. Variation temporelle du peuplement aviaire des écosystème lacustres de la ville de Yamoussoukro, centre de la Côte d'Ivoire. Int J Biol Chem Sci 2016;9:2566.

[18] Sattabongkot J, Suansomjit C, Nguitragool W, Sirichaisinthop J, Warit S, Tiensuwan M, et al. Prevalence of asymptomatic *Plasmodium* infections with sub-microscopic parasite densities in the northwestern border of Thailand: a potential threat to malaria elimination. Malar J 2018;17. https://doi.org/10.1186/s12936-018-2476-1.

[19] Nkoghe D, Akue JP, Gonzalez JP, Leroy EM. Prevalence of *Plasmodium falciparum* infection in asymptomatic rural Gabonese populations. Malar J 2011;10:33. https://doi.org/10.1186/1475-2875-10-33.

[20] Sattabongkot J, Suansomjit C, Nguitragool W, Sirichaisinthop J, Warit S, Tiensuwan M, et al. Prevalence of asymptomatic *Plasmodium* infections with sub-microscopic parasite densities in the northwestern border of Thailand: a potential threat to malaria elimination. Malar J 2018;17:329. https://doi.org/10.1186/s12936-018-2476-1.

[21] Assele V, Ndoh GE, Nkoghe D, Fandeur T. No evidence of decline in malaria burden from 2006 to 2013 in a rural Province of Gabon: implications for public health policy. BMC Publ Health 2015;15. https://doi.org/10.1186/s12889-015-1456-4.

[22] Mourembou G, Nzondo SM, Ndjoyi-Mbiguino A, Lekana-Douki JB, Kouna LC, Matsiegui PB, et al. Co-circulation of *Plasmodium* and Bacterial DNAs in blood of febrile and afebrile children from urban and rural areas in Gabon. Am J Trop Med Hyg 2016;95:123–32. https://doi.org/10.4269/ajtmh.15-0751.

[23] Nankabirwa J, Zurovac D, Njogu JN, Rwakimari JB, Counihan H, Snow RW, et al. Malaria misdiagnosis in Uganda – implications for policy change. Malar J 2009;8:66. https://doi.org/10.1186/1475-2875-8-66.

[24] Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwakasungula E, Mwerinde O, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ 2004;329:1212. https://doi.org/10.1136/bmj.38251.658229.55.

[25] Simonsen PE, Onapa AW, Asio SM. *Mansonella perstans* filariasis in Africa. Acta Trop 2011;120:S109–20. https://doi.org/10.1016/j.actatropica.2010.01.014.

[26] Klion AD, Massougbodji A, Sadeler BC, Ottesen EA, Nutman TB. Loiasis in Endemic and nonendemic populations: immunologically mediated differences in clinical presentation. J Infect Dis 1991;163:1318–25. https://doi.org/10.1093/infdis/163.6.1318.

[27] Bassene H, Sambou M, Fenollar F, Clarke S, Djiba S, Mourembou G, et al. High Prevalence of *Mansonella perstans* filariasis in rural Senegal. Am J Trop Med Hyg 2015;93:601–6. https://doi.org/10.4269/ajtmh.15-0051.

[28] Mourembou G, Fenollar F, Lekana-Douki JB, Ndjoyi Mbiguino A, Maghendji Nzondo S, Matsiegui PB, et al. *Mansonella*, including a potential new species, as common parasites in children in Gabon. PLoS Neglected Trop Dis 2015;9. https://doi.org/10.1371/journal.pntd.0004155.

[29] Asse KV, Seka A, Poe P, Diabate C, Brou-Simonne, Plo KJ. Méningites bactériennes de l'enfant au centre hospitalier universitaire de Bouaké en République de Côte d'Ivoire. Arch Pediatr 2001;8:1266–7. https://doi.org/10.1016/S0929-693X(01)00641-8.

[30] Park SE, Pak GD, Aaby P, Adu-Sarkodie Y, Ali M, Aseffa A, et al. The relationship between invasive nontyphoidal *Salmonella* disease, other bacterial bloodstream infections, and malaria in Sub-Saharan Africa. Clin Infect Dis 2016;62:S23–31. https://doi.org/10.1093/cid/civ893.

[31] Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis 2010;10:417–32. https://doi.org/10.1016/S1473-3099(10)70072-4.

[32] Mediannikov O, Socolovschi C, Million M, Sokhna C, Bassene H, Diatta G, et al. Molecular identification of pathogenic bacteria in eschars from acute febrile patients, Senegal. Am J Trop Med Hyg 2014;91:1015–9. https://doi.org/10.4269/ajtmh.13-0629.

[33] Ndiaye C, Bassene H, Lagier JC, Raoult D, Sokhna C. Asymptomatic carriage of *Streptococcus pneumoniae* detected by qPCR on the palm of hands of populations in rural Senegal. PLoS Neglected Trop Dis 2018;12:e0006945. https://doi.org/10.1371/journal.pntd.0006945.

[34] Berrelha J, Briolant S, Muller F, Rolain JM, Marié JL, Pagés F, et al. *Rickettsia felis* and *Rickettsia massiliae* in ivory coast, Africa. Clin Microbiol Infect 2009;15:251–2. https://doi.org/10.1111/j.1469-0691.2008.02273.x.

[35] Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, Bassene H, et al. *Rickettsia felis*–associated uneruptive fever, Senegal. Emerg Infect Dis 2010;16:1140–2. https://doi.org/10.3201/eid1607.100070.

[36] Richards AL, Jiang J, Omulo S, Dare R, Abdirahman K, Ali A, et al. Human infection with *Rickettsia felis*, Kenya. Emerg Infect Dis 2010;16:1081–6. https://doi.org/10.3201/eid1607.091885.

[37] Mediannikov O, Socolovschi C, Edouard S, Fenollar F, Mouffok N, Bassene H, et al. Common epidemiology of *Rickettsia felis* infection and malaria, Africa. Emerg Infect Dis 2013;19:1775–83. https://doi.org/10.3201/eid1911.130361.

[38] Fenollar F, Lagier JC, Raoult D. *Tropheryma whipplei* and Whipple's disease. J Infect 2014;69:103–12. https://doi.org/10.1016/j.jinf.2014.05.008.

[39] Keita AK, Bassene H, Tall A, Sokhna C, Ratmanov P, Trape JF, et al. *Tropheryma whipplei*: a common bacterium in rural Senegal. PLoS Neglected Trop Dis 2011;5. https://doi.org/10.1371/journal.pntd.0001403.

[40] Vinnemeier CD, Klupp EM, Krumkamp R, Rolling T, Fischer N, Owusu-Dabo E, et al. *Tropheryma whipplei* in children with diarrhoea in rural Ghana. Clin Microbiol Infect 2016;22. https://doi.org/10.1016/j.cmi.2015.09.022. 65.e1-65.e3.

[41] Ramharter M, Harrison N, Bühler T, Herold B, Lagler H, Lötsch F, et al. Prevalence and risk factor assessment of *Tropheryma whipplei* in a rural community in Gabon: a community-based cross-sectional study. Clin Microbiol Infect 2014;20:1189–94. https://doi.org/10.1111/1469-0691.12724.

[42] Fenollar F, Mediannikov O, Socolovschi C, Bassene H, Diatta G, Richet H, et al. *Tropheryma whipplei* bacteremia during fever in rural West Africa. Clin Infect Dis 2010;51:515–21. https://doi.org/10.1086/655677.

[43] Bassene H, Mediannikov O, Socolovschi C, Ratmanov P, Keita AK, Sokhna C, et al. *Tropheryma whipplei* as a cause of epidemic fever, Senegal, 2010–2012. Emerg Infect Dis 2016;22:1229–34. https://doi.org/10.3201/eid2207.150441.

[44] Mourembou G, Fenollar F, Socolovschi C, Lemamy GJ, Nzoughe H, Kouna LC, et al. Molecular detection of fastidious and common bacteria as well as *Plasmodium* spp. in febrile and afebrile children in Franceville, Gabon. Am J Trop Med Hyg 2015;92:926–32. https://doi.org/10.4269/ajtmh.14-0699.

[45] Ramharter M, Harrison N, Bühler T, Herold B, Lagler H, Lötsch F, et al. Prevalence and risk factor assessment of *Tropheryma whipplei* in a rural community in Gabon: a community based cross-sectional study. Clin Microbiol Infect 2014;20:1189–94. https://doi.org/10.1111/1469-0691.12724.

[46] Church J, Maitland K. Invasive bacterial co-infection in African children with *Plasmodium falciparum* malaria: a systematic review. BMC Med 2014;12. https://doi.org/10.1186/1741-7015-12-31.

[47] Mtove G, Amos B, Von Seidlein L, Hendriksen I, Mwambuli A, Kimera J, et al. Invasive salmonellosis among children admitted to a rural Tanzanian hospital and a comparison with previous studies. PloS One 2010;5:e9244. https://doi.org/10.1371/journal.pone.0009244.

[48] Orish VN, Ansong JY, Onyeabor OS, Sanyaolu AO, Oyibo WA, Iriemenam NC. Overdiagnosis and overtreatment of malaria in children in a secondary healthcare centre in Sekondi-Takoradi, Ghana. Trop Doct 2016;46:191–8. https://doi.org/10.1177/0049475515622861.

[49] WHO | WHO informal consultation on fever management in peripheral health care settings: a global review of evidence and practice. WHO; 2013. Available: http://www.who.int/malaria/publications/atoz/9789241506489/en/.