

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, Celia Scherelle Boumbanda Koyo, Landry Doua Bongue, Sébastien Cortaredona, Adèle N'douba Kakou, Djanwai Berenger Konan, Yao Kouassi Patrick, Nadia Amanzougaghene, Jean-David N'guessan, Bernard Davoust, et al.

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Bilé Cyrille Hervé Ehounoud, Celia Scherelle Boumbanda Koyo, Landry Doua Bongue, Sébastien Cortaredona, Adèle N'douba Kakou, et al.. Assessment of the burden of malaria and bacteraemia by retrospective molecular diagnosis in febrile illnesses and first-line anti-infectives in Côte d'Ivoire. Travel Medicine and Infectious Disease, 2021, 43, pp.102105. 10.1016/j.tmaid.2021.102105. hal-03429328

HAL Id: hal-03429328 https://amu.hal.science/hal-03429328

Submitted on 2 Aug 2023

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- 1 Assessment of the burden of malaria and bacteraemia by retrospective molecular
- 2 diagnosis in febrile illnesses and first-line anti-infectives in Côte d'Ivoire
- 3 Bilé Cyrille Hervé EHOUNOUD^{a,b,c,d£†}, Celia Scherelle BOUMBANDA KOYO^{a,b,e,f£}, Landry
- 4 DOUA BONGUE^g, Sébastien CORTAREDONA^{a,b}, Adèle N'DOUBA KAKOU^h, Djanwai
- 5 Berenger KONAN^g, Patrick YAO KOUASSI^c, Nadia AMANZOUGAGHENE^{b,i}, Jean-David
- 6 N'GUESSAN^c, Bernard DAVOUST^{b,i}, Didier RAOULT^{b,i}, Oleg MEDIANNIKOV^{b,i},
- 7 Florence FENOLLAR^{a,b*}
- 8 [£]Equally contributed
- 9 ^aAix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France
- 10 ^bIHU-Méditerranée Infection, Marseille, France
- 11 °Félix Houphouët Boigny Université, UFR Biosciences, Côte D'Ivoire
- 12 ^dInstitut Pierre Richet, Institut National de Santé Publique, Bouaké, Côte d'Ivoire
- 13 ^eUnité d'Evolution, Epidémiologie et Résistances Parasitaires (UNEEREP), Centre
- 14 International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon
- ¹⁵ ^fEcole Doctorale Régionale en Infectiologie Tropicale d'Afrique Centrale, Franceville, Gabon
- 16 ^gCentre Médico Social Wale, BP 1336 Yamoussoukro, Côte d'Ivoire
- 17 ^hFaculté de Médecine, Pharmacie et Odonto-Stomatologie, Université Félix Houphouët
- 18 Boigny, Côte d'Ivoire
- ¹⁹ ⁱAix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France
- 20 *Corresponding author: Florence FENOLLAR (florence.fenollar@univ-amu.fr)
- 21 [†] Doctor Bilé Cyrille Hervé EHOUNOUD passed away in August 2020
- 22 Word abstract count: 248
- Word text count: 3,341
- 24 Keywords: Fever; Bacteraemia; Malaria; Côte d'Ivoire; Salmonella enterica; Streptococcus
- 25 pneumoniae

27 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.

30 Methods: Blood samples from 438 febrile and 346 afebrile people were screened using
31 molecular tools.

32 **Results:** *Plasmodium falciparum* was the most common microorganism associated with fever

33 (46.8% in febrile; 23.4% in afebrile people p < 0.001). Bacteraemia was detected in 21.7% of

34 febrile people and 12.7% of afebrile people (*p*=0.001). *Streptococcus pneumoniae* was the

35 main cause of bacteraemia (7.1% of febrile and 0.6% of afebrile individuals; p < 0.001). Non-

36 typhoidal *Salmonella* spp. was detected in 4.5% of febrile people and 1.2% of afebrile

37 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%),

39 Streptococcus pyogenes (0.7%), and Plasmodium ovale (4.6%). The prevalence in febrile and

40 afebrile people was similar for *Staphylococcus aureus* (3.6-4.9%), *Rickettsia felis* (5.5-6.4%),

41 *Mansonella perstans* (3.0-3.2%), and *Plasmodium malariae* (1.6-2.3%). Comorbidities were

42 higher in febrile than in afebrile subjects (10.3% versus 5.5%; p=0.01); 82% involving P.

43 *falciparum*. All patients co-infected with *P. falciparum* and *S. pneumoniae* were febrile

44 whereas 30.0% of those infected by *P. falciparum* alone were not (*p*=0.02). Among febrile

45 participants, 30.4% with malaria and 54.7% with bacteraemia had received neither

46 antimalarial nor antibiotic therapy.

47 **Conclusion:** Identification of etiologies of acute febrile diseases in sub-Saharan Africa

48 proposes keys to successful treatment and prevention of infectious diseases. Vaccination

49 campaigns may decrease the morbidity of mono- and co-infections by preventable

50 microorganisms.

- 52 **1. Introduction**
- 53

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.

60 The burden of bacterial aetiologies is poorly understood. Traditional diagnostic tools, 61 such as blood cultures, are available almost exclusively in large cities. Furthermore, blood 62 cultures do not allow the isolation of intracellular or highly fastidious bacteria. Only common 63 bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Salmonella* spp. are 64 regarded as the main bacterial aetiologies of fever [6,7]. Recently, molecular strategies for the 65 characterisation of bacterial bloodstream infections enabled to highlight the implication of 66 fastidious bacteria such as Rickettsia felis and Tropheryma whipplei in Senegal and Gabon 67 [8,9].

68 In Côte d'Ivoire between 1996 and 1998, studies investigating bacteraemia among 69 febrile HIV-infected patients identified non-typhoidal Salmonella enterica (15), S. 70 pneumoniae (13), Escherichia coli (6), Shigella spp. (2), S. aureus (2), Proteus mirabilis (1), 71 and Salmonella enterica Typhi (1) as bacterial aetiologies [10]. More recently, between 2012 72 and 2014, after a long period when culture equipment was unavailable, a study on the 73 morbidity related to bacterial blood infections was conducted in Bouaké (central Côte 74 d'Ivoire) with Klebsiella pneumoniae and S. enterica being involved in 94% of bloodstream 75 infections followed by S. aureus [11].

76	Although there is evidence of the circulation of zoonotic bacteria such as C. burnetii,
77	Rickettsia spp., Borrelia spp., and Anaplasma spp. in cattle, sheep, goats, and ticks in Côte
78	d'Ivoire [12,13], their role in human febrile illness has not yet been thoroughly investigated.
79	Our study, therefore, aimed to determine the burden of malaria, Mansonellosis, common
80	and fastidious bacteria in Yamoussoukro (Côte d'Ivoire) using specific molecular tools and to
81	compare the accuracy of treatments prescribed in the hospital and the retrospective molecular
82	diagnosis.
83	
84	2. Material and methods
85	
86	2.1. Ethics statement
87	The Côte d'Ivoire National Research Ethics Committee and the Wale Hospital of
88	Yamoussoukro (agreement number 86 MSLS / CNER-dkn) approved the study protocol.
89	Written informed consent was obtained from each adult subject and from the parents or legal
90	guardians of children included in the study.
91	2.2. Study site
92	The study was carried out at the Wale hospital in Yamoussoukro in Côte d'Ivoire (West
93	Africa). Yamoussoukro is the political and administrative capital of the country and is located
94	248 km north of Abidjan, the economic capital (Figure 1). In the district, the vegetation is
95	dominated by wet savanna with varying small trees. This area is characterised by its mean
96	temperature of 26°C and the rainy season peaks between March-July, and September-
97	October. The district of Yamoussoukro has a total of 362,000 inhabitants, of whom 199,100
98	(55%) live in urban areas.
99	2.3. Study population and sample collection

100 This is a cross-sectional epidemiological study. Between September 2014 and April 101 2015, 438 people consulting at the hospital for fever (axillary temperature $\geq 37.5^{\circ}$ C) were 102 recruited as well as comparative group of 346 asymptomatic people recruited at the same 103 time. The afebrile comparative group consisted of asymptomatic volunteers, parents and 104 children accompanying people seeking medical care, and who did not have episodes of fever 105 in the previous month.

106 Three drops of blood from each participant (about 200 μ L) were collected using the 107 finger prick method and collected in an Eppendorf tube containing 20 μ L of citrate. On site, 108 DNA was extracted with QIAamp DNA kit (Qiagen, Hilden, Germany) in line with the 109 manufacturer's protocol without elution. The columns of DNA were stored in the refrigerator 110 before being transferred to the Institut Hospitalo-Universitaire Mediterranée Infection 111 (Marseille, France), where DNA elution was performed with 200 μ L of buffer AE (Acetone, 112 EDTA). DNA was stored at +4°C until used for PCR analysis.

113

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.

120 The final reaction volume (20 μ L) of qPCR contained 10 μ L of Master mix No ROX 121 (Eurogentec, Liege, Belgium), 3.5 μ L of distilled water (DNAase/RNAase free), 2.5 μ M of 122 probe, 20 μ M of each primer, and 5 μ L DNA. All reactions were carried using a CFX 96 123 (Bio-Rad, Marnes-la-Coquette, France) according to following settings: DNA denaturation 124 steps at 50°C for 2 min and 95°C for 5 min followed by 40 cycles of one second at 95°C, 35 seconds at 60°C, and an extension step for 30 seconds 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.

128

2.5. Statistical analysis

129 Statistical analyses were performed using SAS university edition software (version 9.4, 130 SAS Institute Inc., Cary, NC, USA) and R software (version 3.6.2). Chi-square's and 131 Fischer's tests were used to compare frequencies between febrile and afebrile subjects. For 132 statistical significance, the two-sided *p. values* were set at 0.005. Principal Component 133 Analysis (PCA) was performed to assess the relationship between relevant pathogens, 134 demographic characteristics, fever, and other clinical signs.

135

136 **3. Results**

137

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

141 The quality of the DNA extracted from all blood samples was satisfactory, given that 142 the cycle threshold values (Ct) of qPCR targeting human β -actin gene ranged from 19 to 26. 143 The demographic and clinical characteristics of the population studied are summarised in 144 Table 1. Principal Component Analysis showed that *P. falciparum* and *S. pneumoniae* were 145 strongly correlated with febrile status, asthenia, cough, rhinorrhoea, headaches, vomiting, and 146 nausea (Figure 2). Otherwise, non-typhoidal *Salmonella, S. enterica* Paratyphi, diarrhoea, and 147 abdominal pain were strongly correlated.

- **3.1. Parasitaemia**
- 149 **3.1.1.** Malaria

Plasmodium parasites were the more frequently detected microorganisms with an 150 151 overall prevalence of 38.5% (302/784). They were also significantly higher in febrile (49.5%, 152 217/438) than in afebrile subjects (24.6%, 85/346; p<0.001) (Table 2). Among febrile 153 patients, P. falciparum was more frequently detected in rural (62.1%, 72/116) than in urban 154 subjects (45%, 145/322; p<0.001), while no statistical differences were observed between 155 rural and urban areas for *P. ovale* and *P. malariae* (Table 2). *P. falciparum* represented 94.7% 156 (286/302) of samples which were positive for *Plasmodium* species. Its prevalence was 157 significantly higher in febrile (46.8%, 205/438) than in afebrile subjects (23.4%, 81/346; 158 p<0.0001). P. ovale was only identified in febrile patients (4.6%, 20/438). P. malariae was 159 less common in febrile patients (1.6%, 7/438) than in afebrile subjects (2.3%, 8/346; 160 p=0.468). In febrile people, the prevalence of malaria due to *P. falciparum* decreased with age 161 (p<0.001) (Figure 3, Table 3). For P. malariae and P. ovale, no statistical differences were 162 observed between age groups (Table 3).

163

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 (Figure 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).

169

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

178 3). The two afebrile people who were positive for *S. pneumoniae* were 22 and 23 years old.

179 Salmonella spp. was more frequently identified in febrile subjects (7.5%, 33/438) than 180 in afebrile subjects (1.2%, 4/346, p < 0.001). Non-typhoidal Salmonella was identified only in 181 afebrile people (1.2%, 4/346) but was significantly associated with fever (p < 0.001). Indeed, 182 non-typhoidal Salmonella was the most frequently detected species in febrile subjects (4.5%, 183 18/438), followed by Salmonella enterica Paratyphi (2.1%, 9/438), and Salmonella enterica 184 Typhi (1.4%, 6/438). No significant difference was observed between febrile people living in 185 rural and urban areas for non-typhoidal Salmonella. and S. enterica Paratyphi (Table 2) while 186 S. enterica Typhi was significantly higher in rural areas (3.5%, 4/116) compared to urban 187 areas (0.6%, 2/322; p=0.045). For non-typhoidal Salmonella and S. enterica Paratyphi, no 188 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). 189

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 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.

206

3.4. Treatment prescribed at hospital and retrospective molecular diagnostic

207 Treatments prescribed at the hospital were compared to retrospective molecular 208 diagnosis. Of the 95 patients with bacteraemia, 52 did not received antibiotic treatment. Of 209 them, 15 were infected with R. felis, 10 with S. pneumoniae, 7 with non-typhoidal Salmonella, 210 6 with S. aureus, 3 with S. enterica Paratyphi, one with S. enterica Typhi, and one with S. 211 pyogenes. Nine of the 52 patients who did not receive an antibiotic prescription had a mixed 212 bacterial infection. One of them were co-infected with R. felis and S. aureus, one with R. felis 213 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 214 215 and S. pneumoniae. Forty-three of the 95 patients with bacteraemia received antibiotics. 216 Retrospective molecular diagnosis of bacteraemia and antibiotics prescribed when febrile 217 patients were admitted to hospital are summarised in Table 5. Conversely, 133 of 343 patients 218 without bacteraemia linked with screened bacteria received antibiotics (39 combinations of 219 amoxicillin and clavulanic acid, 35 amoxicillin, one combination of amoxicillin, clavulanic 220 acid and ampicillin, 11 cefadroxil, 12 cefpodoxime, 3 azithromycin, 16 ciprofloxacin, one 221 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 (Figure 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
(Figure 4). Of these, 109 received artemether-lumefantrine, one received artesunateamodiaquine, 3 quinine, 2 sulfadoxine-pyrimethamine, and one received piperaquine.

228

4. Discussion

230

231 Malaria is the main aetiology in patients consulting for fever at the Wale Hospital in 232 Yamoussoukro, with P. falciparum being the most frequently identified species. Previous 233 studies in Côte d'Ivoire were performed using the thick smear procedure, which is known to 234 be less sensitive than molecular tools. In 2008, the prevalence of malaria at the Abobo 235 Hospital in Abidjan was estimated at 29.5% among 902 febrile people and 13.5% among 681 236 afebrile people [15]. However, in the town of Tabou in 2010 and 2011 the prevalence of 237 malaria was estimated at between 55.1% of 147 and 67.1% of 158 febrile people, and between 238 45% of 948 to 56.8% of 1,029 afebrile people, respectively) [16]. In addition to the difference 239 of sensitivity of each tool, geographic differences could explain the disparity of malaria 240 prevalence, as Yamoussoukro alternates between forest and savannah [17] while Abobo is in 241 a plateau area and Tabou is predominantly equatorial forest. Overall, a high rate of 242 asymptomatic malaria was observed, as has been reported in other countries with high malaria 243 transmission [18,19]. This high number of asymptomatic infections poses a challenge to 244 malaria elimination efforts [20]. Finally, malaria was more prevalent among younger people 245 and in rural areas, as previously reported [1,16,21-24]. The prevalence of *M. perstans* was 246 similar in febrile and afebrile subjects. Indeed, M. perstans is quite often asymptomatic in 247 populations in endemic areas [25]. Conversely, *M. perstans* may be responsible for invasive 248 infections for people from non-endemic areas coming to endemic areas in Africa or America 249 [25,26]. As already observed, *M. perstans* is significantly more common among adult and

rural populations [27,28].

251 Bacteraemia was found in 21.7% of patients, with S. pneumoniae being the leading 252 aetiological agent and strongly associated with fever. A first study on the morbidity of 253 bloodstream infections, conducted using blood cultures between 2012 and 2014 in Côte 254 d'Ivoire, found similar bacteraemia rates (22.5%, 293) but did not find S. pneumoniae [11]. 255 Nevertheless, based on a previous study carried out in Côte d'Ivoire between 1999 and 2013, 256 on aetiologies of meningitis among children, S. pneumoniae was responsible for 44% of 257 meningitis cases [29]. Although S. pneumoniae is the main cause of febrile bacteraemia, no 258 fatal cases were observed in our study.

S. enterica Typhi and Paratyphi were only observed in febrile people while nontyphoidal *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].

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].

287 Co-infections between malaria and bacterial bloodstream infections, mainly due to non-288 typhoid Salmonella, are commonly described in sub-Saharan Africa in febrile people and are 289 associated with an increased risk of poor prognosis [46,47]. Surprisingly, for the first time we 290 report a co-infection between P. falciparum and non-typhoid Salmonella in 3 afebrile children 291 who had not experienced fever nor taken antibiotic or antimalarial treatment in the previous 292 month. Of course, the molecular analysis used to screen for pathogens cannot provide any 293 information on the viability of these microorganisms but the persistence of bacterial DNA in 294 the blood has never been reported for acute infections, to the best of our knowledge. In 295 addition, it should be noted that all patients co-infected with S. pneumoniae and P. falciparum 296 were febrile. This suggests the further necessity to evaluate the efficacy of the vaccination 297 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 300 reliable diagnostic tools available. A Ghanaian study found that 4.5% of the 247 children with 301 a retrospectively confirmed diagnosis of malaria had not received antimalarial drugs, while up 302 to 84.1% of the 446 children without malaria had received antimalarial drugs [48]. It is likely 303 that the inappropriate use of anti-infectives is still common practice in sub-Saharan Africa. 304 Indeed, the diversity of infectious aetiologies, the presence of co-infections, the prevalence of 305 asymptomatic infections, the few or complete lack of diagnostic tools make the therapeutic choices available to physicians very difficult. Current WHO recommendations for the 306 307 management of fever in sub-Saharan Africa are to identify signs of severity in patients with 308 fever. If there are signs of seriousness, antimalarials and antibiotics are automatically 309 prescribed before admission. After admission, rapid diagnostic testing for malaria and blood 310 film are performed. If at least one test is positive, parenteral antimalarials should be given, 311 and in other cases parenteral antibiotics should be administered before assessing for other 312 causes of fever. If there is no sign of severity, a rapid malaria diagnostic test and a blood 313 smear should be performed, and if at least one of them is positive, antimalarial treatments 314 should be administered. In other cases, an antimalarial treatment should be prescribed and the 315 patient must be monitored before trying to identify another cause of fever [49].

316 Improving the management of febrile illnesses in sub-Saharan Africa requires effort and 317 resources that go beyond the deployment of rapid malaria tests. Indeed, it is also necessary to 318 understand the repertoire of bacteria present in the ecosystems of the different regions in order 319 to simultaneously answer the question that if it is not malaria, then what can it be, and which 320 empirical antibiotic therapy would be the most appropriate. Although our study has some 321 limitations (lack of traditional blood cultures and lack of data about antibiotic susceptibility, 322 differences in age/sex in febrile group of patients and comparative afebrile group, lack of data 323 on RNA viruses as causative agents due to the logistic difficulties for the RNA conservation), 324 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.

330 **References**

- 1. Cotter C, Sturrock HJW, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing
- 332 epidemiology of malaria elimination: new strategies for new challenges. Lancet
- 333 2013;382:900–11. doi:10.1016/S0140-6736(13)60310-4
- 2. Kouassi BL, de Souza DK, Goepogui A, Balde SM, Diakité L, Sagno A, et al. Low
- 335 prevalence of *Plasmodium* and absence of malaria transmission in Conakry, Guinea:
- 336 prospects for elimination. Malar J 2016;15:175. doi:10.1186/s12936-016-1230-9
- 337 3. WHO | World Malaria Report 2013. Available:
- 338 https://www.who.int/malaria/publications/world_malaria_report_2013/en/
- 339 4. Chipwaza B, Mhamphi GG, Ngatunga SD, Selemani M, Amuri M, Mugasa JP, et al.
- 340 Prevalence of bacterial febrile illnesses in children in Kilosa district, Tanzania. PLoS Negl
- 341 Trop Dis 2015;9:e0003750. doi:10.1371/journal.pntd.0003750
- 342 5. Chipwaza B, Mugasa JP, Mayumana I, Amuri M, Makungu C, Gwakisa PS.
- 343 Community knowledge and attitudes and health workers' practices regarding non-malaria
- 344 febrile illnesses in eastern Tanzania. PLoS Negl Trop Dis 2014;8:e2896.
- 345 doi:10.1371/journal.pntd.0002896
- 346 6. Msemo OA, Mbwana J, Mahende C, Malabeja A, Gesase S, Crump JA, et al.
- 347 Epidemiology and antimicrobial susceptibility of *Salmonella enterica* bloodstream isolates
- 348 among febrile children in a rural district in Northeastern Tanzania: A Cross-sectional study.
- 349 Clin Infect Dis 2019;68:S177–S182. doi:10.1093/cid/ciy1126
- 350 7. Msefula CL, Olgemoeller F, Jambo N, Segula D, Van Tan T, Nyirenda TS, et al.
- 351 Ascertaining the burden of invasive *Salmonella* disease in hospitalised febrile children aged
- under four years in Blantyre, Malawi. PLoS Negl Trop Dis 2019;13:e0007539. doi:
- 353 10.1371/journal.pntd.0007539
- 8. Mourembou G, Lekana-Douki JB, Mediannikov O, Nzondo SM, Kouna LC, Essone

355 JCBB, et al. Possible role of *Rickettsia felis* in acute febrile illness among children in Gabon.

356 Emerg Infect Dis 2015;21:1808–15. doi:10.3201/eid2110.141825

357 9. Sokhna C, Mediannikov O, Fenollar F, Bassene H, Diatta G, Tall A, et al. Point-of-

358 care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl Trop Dis 2013;7:e1999.

359 doi:10.1371/journal.pntd.0001999

360 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.

362 AIDS 2002;16:909–18. doi: 10.1097/00002030-200204120-00011

363 11. Akoua-Koffi C, Tia H, Plo JK, Monemo P, Cissé A, Yao C, et al. Epidemiology of

364 community-onset bloodstream infections in Bouaké, central Côte d'Ivoire. New Microbes

365 New Infect 2015;7:100–4. doi:10.1016/j.nmni.2015.06.009

366 12. Ehounoud CB, Yao KP, Dahmani M, Achi YL, Amanzougaghene N, Kacou N'Douba

367 A, et al. Multiple pathogens including potential new species in tick vectors in Côte d'Ivoire.

368 PLoS Negl Trop Dis 2016;10:e0004367. doi:10.1371/journal.pntd.0004367

369 13. Kanouté YB, Gragnon BG, Schindler C, Bonfoh B, Schelling E. Epidemiology of

370 brucellosis, Q Fever and Rift Valley Fever at the human and livestock interface in northern

371 Côte d'Ivoire. Acta Trop 2017;165:66–75. doi:10.1016/j.actatropica.2016.02.012

372 14. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Bassene H, Molez JF, et al.

373 Coxiella burnetii in humans and ticks in rural Senegal. PLoS Negl Trop Dis 2010;4:e654.

374 doi:10.1371/journal.pntd.0000654

375 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.

378 16. Bassa FK, Ouattara M, Silué KD, Adiossan LG, Baikoro N, Koné S, et al.

379 Epidemiology of malaria in the Taabo health and demographic surveillance system, south-

- 380 central Côte d'Ivoire. Malar J 2016;15. doi:10.1186/s12936-015-1076-6
- 381 17. Konan E, Yaokokore-Beibro H. Variation temporelle du peuplement aviaire des
- 382 écosystème lacustres de la ville de Yamoussoukro, centre de la Côte d'Ivoire. Int J Biol Chem
- 383 Sci 2016;9:2566.
- 18. Sattabongkot J, Suansomjit C, Nguitragool W, Sirichaisinthop J, Warit S, Tiensuwan
- 385 M, et al. Prevalence of asymptomatic *Plasmodium* infections with sub-microscopic parasite
- 386 densities in the northwestern border of Thailand: a potential threat to malaria elimination.
- 387 Malar J 2018;17. doi:10.1186/s12936-018-2476-1
- 388 19. Nkoghe D, Akue JP, Gonzalez JP, Leroy EM. Prevalence of *Plasmodium falciparum*
- 389 infection in asymptomatic rural Gabonese populations. Malar J 2011;10:33.
- 390 doi:10.1186/1475-2875-10-33
- 391 20. Sattabongkot J, Suansomjit C, Nguitragool W, Sirichaisinthop J, Warit S, Tiensuwan
- 392 M, et al. Prevalence of asymptomatic *Plasmodium* infections with sub-microscopic parasite
- 393 densities in the northwestern border of Thailand: a potential threat to malaria elimination.
- 394 Malar J 2018;17:329. doi:10.1186/s12936-018-2476-1
- 395 21. Assele V, Ndoh GE, Nkoghe D, Fandeur T. No evidence of decline in malaria burden
- 396 from 2006 to 2013 in a rural Province of Gabon: implications for public health policy. BMC
- 397 Public Health 2015;15. doi:10.1186/s12889-015-1456-4
- 398 22. Mourembou G, Nzondo SM, Ndjoyi-Mbiguino A, Lekana-Douki JB, Kouna LC,
- 399 Matsiegui PB, et al. Co-circulation of *Plasmodium* and Bacterial DNAs in blood of febrile
- 400 and afebrile children from urban and rural areas in Gabon. Am J Trop Med Hyg 2016;95:
- 401 123–32. doi:10.4269/ajtmh.15-0751
- 402 23. Nankabirwa J, Zurovac D, Njogu JN, Rwakimari JB, Counihan H, Snow RW, et al.
- 403 Malaria misdiagnosis in Uganda implications for policy change. Malar J 2009;8:66.
- 404 doi:10.1186/1475-2875-8-66

- 405 24. Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwakasungula E, Mwerinde O, et al.
- 406 Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective
- 407 study. BMJ 2004;329:1212. doi:10.1136/bmj.38251.658229.55
- 408 25. Simonsen PE, Onapa AW, Asio SM. Mansonella perstans filariasis in Africa. Acta
- 409 Trop 2011;120:S109–S120. doi:10.1016/j.actatropica.2010.01.014
- 410 26. Klion AD, Massougbodji A, Sadeler BC, Ottesen EA, Nutman TB. Loiasis in Endemic
- 411 and nonendemic populations: Immunologically mediated differences in clinical presentation. J
- 412 Infect Dis 1991;163:1318–25. doi:10.1093/infdis/163.6.1318
- 413 27. Bassene H, Sambou M, Fenollar F, Clarke S, Djiba S, Mourembou G, et al. High
- 414 Prevalence of *Mansonella perstans* filariasis in rural Senegal. Am J Trop Med Hyg 2015;93:
- 415 601–6. doi:10.4269/ajtmh.15-0051
- 416 28. Mourembou G, Fenollar F, Lekana-Douki JB, Ndjoyi Mbiguino A, Maghendji
- 417 Nzondo S, Matsiegui PB, et al. Mansonella, including a potential new species, as common
- 418 parasites in children in Gabon. PLoS Negl Trop Dis 2015;9.
- 419 doi:10.1371/journal.pntd.0004155
- 420 29. Asse KV, Seka A, Poe P, Diabate C, Brou-Simonne, Plo KJ. Méningites bactériennes
- 421 de l'enfant au centre hospitalier universitaire de Bouaké en République de Côte d'Ivoire. Arch
- 422 Pédiatrie 2001;8:1266–7. doi:10.1016/S0929-693X(01)00641-8
- 423 30. Park SE, Pak GD, Aaby P, Adu-Sarkodie Y, Ali M, Aseffa A, et al. The relationship
- 424 between invasive nontyphoidal *Salmonella* disease, other bacterial bloodstream infections,
- 425 and malaria in Sub-Saharan Africa. Clin Infect Dis 2016;62:S23–S31. doi:10.1093/cid/civ893
- 426 31. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in
- 427 Africa: a systematic review and meta-analysis. Lancet Infect Dis 2010;10:417–32.
- 428 doi:10.1016/S1473-3099(10)70072-4
- 429 32. Mediannikov O, Socolovschi C, Million M, Sokhna C, Bassene H, Diatta G, et al.

- 430 Molecular identification of pathogenic bacteria in eschars from acute febrile patients, Senegal.
- 431 Am J Trop Med Hyg 2014;91:1015–9. doi: 10.4269/ajtmh.13-0629
- 432 33. Ndiaye C, Bassene H, Lagier JC, Raoult D, Sokhna C. Asymptomatic carriage of
- 433 Streptococcus pneumoniae detected by qPCR on the palm of hands of populations in rural
- 434 Senegal. PLoS Negl Trop Dis 2018;12:e0006945. doi: 10.1371/journal.pntd.0006945
- 435 34. Berrelha J, Briolant S, Muller F, Rolain JM, Marié JL, Pagés F, et al. Rickettsia felis
- 436 and *Rickettsia massiliae* in Ivory Coast, Africa. Clin Microbiol Infect 2009;15:251–2.
- 437 doi:10.1111/j.1469-0691.2008.02273.x
- 438 35. Socolovschi C, Mediannikov O, Sokhna C, Tall A, Diatta G, Bassene H, et al.
- 439 *Rickettsia felis*-associated uneruptive fever, Senegal. Emerg Infect Dis 2010;16:1140–2.
- 440 doi:10.3201/eid1607.100070
- 441 36. Richards AL, Jiang J, Omulo S, Dare R, Abdirahman K, Ali A, et al. Human infection
- 442 with *Rickettsia felis*, Kenya. Emerg Infect Dis 2010;16:1081–6. doi:10.3201/eid1607.091885
- 443 37. Mediannikov O, Socolovschi C, Edouard S, Fenollar F, Mouffok N, Bassene H, et al.
- 444 Common epidemiology of *Rickettsia felis* infection and malaria, Africa. Emerg Infect Dis
- 445 2013;19:1775-83. doi:10.3201/eid1911.130361
- 446 38. Fenollar F, Lagier JC, Raoult D. *Tropheryma whipplei* and Whipple's disease. J Infect
- 447 2014;69:103–12. doi: 10.1016/j.jinf.2014.05.008
- 448 39. Keita AK, Bassene H, Tall A, Sokhna C, Ratmanov P, Trape JF, et al. Tropheryma
- 449 *whipplei*: A common bacterium in rural Senegal. PLoS Negl Trop Dis 2011;5.
- 450 doi:10.1371/journal.pntd.0001403
- 451 40. Vinnemeier CD, Klupp EM, Krumkamp R, Rolling T, Fischer N, Owusu-Dabo E, et
- 452 al. *Tropheryma whipplei* in children with diarrhoea in rural Ghana. Clin Microbiol Infect
- 453 2016;22:65.e1-65.e3. doi:10.1016/j.cmi.2015.09.022
- 454 41. Ramharter M, Harrison N, Bühler T, Herold B, Lagler H, Lötsch F, et al. Prevalence

- 455 and risk factor assessment of *Tropheryma whipplei* in a rural community in Gabon: a
- 456 community-based cross-sectional study. Clin Microbiol Infect 2014;20:1189–94.
- 457 doi:10.1111/1469-0691.12724
- 458 42. Fenollar F, Mediannikov O, Socolovschi C, Bassene H, Diatta G, Richet H, et al.
- 459 *Tropheryma whipplei* bacteremia during fever in rural West Africa. Clin Infect Dis 2010;51:
- 460 515–21. doi:10.1086/655677
- 461 43. Bassene H, Mediannikov O, Socolovschi C, Ratmanov P, Keita AK, Sokhna C, et al.
- 462 *Tropheryma whipplei* as a cause of epidemic fever, Senegal, 2010–2012. Emerg Infect Dis
- 463 2016;22:1229–34. doi:10.3201/eid2207.150441
- 464 44. Mourembou G, Fenollar F, Socolovschi C, Lemamy GJ, Nzoughe H, Kouna LC, et al.
- 465 Molecular detection of fastidious and common bacteria as well as *Plasmodium* spp. in febrile
- 466 and afebrile children in Franceville, Gabon. Am J Trop Med Hyg 2015;92:926–32. doi:
- 467 10.4269/ajtmh.14-0699
- 468 45. Ramharter M, Harrison N, Bühler T, Herold B, Lagler H, Lötsch F, et al. Prevalence
- 469 and risk factor assessment of *Tropheryma whipplei* in a rural community in Gabon: a
- 470 community based cross-sectional study. Clin Microbiol Infect 2014;20:1189–94. doi:
- 471 10.1111/1469-0691.12724
- 472 46. Church J, Maitland K. Invasive bacterial co-infection in African children with
- 473 Plasmodium falciparum malaria: a systematic review. BMC Med 2014;12. doi:10.1186/1741-
- 474 7015-12-31
- 475 47. Mtove G, Amos B, Von Seidlein L, Hendriksen I, Mwambuli A, Kimera J, et al.
- 476 Invasive salmonellosis among children admitted to a rural Tanzanian hospital and a
- 477 comparison with previous studies. PloS One 2010;5:e9244. doi:
- 478 10.1371/journal.pone.0009244
- 479 48. Orish VN, Ansong JY, Onyeabor OS, Sanyaolu AO, Oyibo WA, Iriemenam NC.

- 480 Overdiagnosis and overtreatment of malaria in children in a secondary healthcare centre in
- 481 Sekondi-Takoradi, Ghana. Trop Doct 2016;46:191–98. doi:10.1177/0049475515622861
- 482 49. WHO | WHO informal consultation on fever management in peripheral health care
- 483 settings: A global review of evidence and practice. WHO 2013. Available:
- 484 http://www.who.int/malaria/publications/atoz/9789241506489/en/

486 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.

- 494 **Declaration of competing interest**
- 495 The authors report no potential conflicts.

496 Legends of figures and tables.

- 497 **Figure 1.** Map of the study site in Côte d'Ivoire.
- 498 Figure 2. Principal Component Analysis (PCA) of clinical and biological outcomes,
- 499 demographic characteristics of 348 febrile patients and 346 afebrile people.
- 500 Figure 3. Prevalence of microorganisms detected among the 438 febrile patients according to
- 501 age.
- 502 Figure 4. Rate of antimalarial (A) and antibiotic (B) prescriptions upon admission to hospital
- 503 compared to retrospective molecular diagnostic outcomes.
- 504 **Table 1.** Demographic and clinical characteristics of the population studied.
- 505 **Table 2.** Prevalence of microorganisms detected in 438 febrile patients and 346 afebrile
- 506 people (A) and their prevalence by geographic origin in febrile patients (B).
- 507 **Table 3.** Prevalence of microorganisms according to age group in febrile people.
- 508 **Table 4.** Prevalence of co-infections among 438 febrile and 346 afebrile people.
- 509 **Table 5.** Retrospective molecular diagnosis of bacteremia and respective antibiotic treatment
- 510 prescribed at admission to hospital in febrile patients.
- 511



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



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



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



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

	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	

Table 1. Demographic and clinical characteristics of the population studied.

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

	438 febrile	346 afebrile	
A.	patients	people	
Microorganisms	Number	of positive (%)	<i>p</i> . 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

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).

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*).

	322 febrile urban	116 febrile rural	
B.	inhabitants	inhabitants	
Microorganisms	Number of positive (%)		<i>p</i> . 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

	Age groups (Years)			-
	<5	5 - 20	>20	-
	Numl	ber of febrile pe	ople	-
	126	123	189	-
Microorganisms	Num	ber of positive (%)	<i>p</i> . 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

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

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

-	Number of	positive (%)	<i>p</i> . 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 4. Prevalence of co-infections among 438 febrile and 346 afebrile people.

438 febrile patients 346 afebrile people

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

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