

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**

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26

27 **ABSTRACT**

28 **Background:** The aetiologies of fever are poorly understood in sub-Saharan Africa. We
29 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
38 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.

51

52 **1. Introduction**

53

54 Despite a substantial decrease in malaria burden in several areas of sub-Saharan Africa
55 [1–3], there is a lack of knowledge around non-malaria febrile illnesses in many countries in
56 this area. In addition, the overlapping of clinical signs between malaria and other febrile
57 illnesses makes differential diagnosis of fever difficult in resource-limited settings [4,5]. The
58 establishment of the local epidemiology of fever aetiologies is critical in order to optimise the
59 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 $\geq 37.5^{\circ}\text{C}$) 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 μL) were collected using the
107 finger prick method and collected in an Eppendorf tube containing 20 μL 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 Méditerranée Infection
111 (Marseille, France), where DNA elution was performed with 200 μL of buffer AE (Acetone,
112 EDTA). DNA was stored at $+4^{\circ}\text{C}$ until used for PCR analysis.

113 **2.4. Assessment of DNA quality and molecular analysis**

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

120 The final reaction volume (20 μL) of qPCR contained 10 μL of Master mix No ROX
121 (Eurogentec, Liege, Belgium), 3.5 μL of distilled water (DNAase/RNAase free), 2.5 μM of
122 probe, 20 μM of each primer, and 5 μL 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

125 seconds at 60°C, and an extension step for 30 seconds at 45°C. In each reaction, two positive
126 controls (DNA of targeted microorganism) and two negative controls (mix alone) were used
127 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

138 The average age of study participants was 20+/-12-year-old (range 3 weeks to 71 years
139 old). Three hundred and forty-four febrile patients were urban residents and 94 came from
140 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.

148 **3.1. Parasitaemia**

149 **3.1.1. Malaria**

150 *Plasmodium* parasites were the more frequently detected microorganisms with an
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***

164 Filariasis due to *M. perstans* was detected in 3.1% (20/784) of subjects, including 3.0%
165 (13/438) of febrile and 3.2% (11/346; $p=0.865$) of afebrile individuals (Figure 3). In febrile
166 patients, the prevalence of *M. perstans* was significantly higher among those living in rural
167 areas (6%, 7/116) as opposed to urban areas (1%, 6/322; $p=0.023$). The prevalence of *M.*
168 *perstans* also increased significantly with age in febrile subjects (Table 3).

169 **3.2. Bacteraemia**

170 Overall, 21.7% of febrile people (95/438) had bacteraemia versus 12.7% of afebrile
171 people (44/346; $p=0.001$). *S. pneumoniae* was the second most frequently detected
172 microorganism, detected in 4.2% (33/784) of positive subjects. The prevalence of *S.*
173 *pneumoniae* was significantly higher in febrile people (7.1%, 31/438) than in afebrile people
174 (0.6%, 2/346; $p<0.001$). No significant difference was observed between febrile people living

175 in urban or rural areas (Table 2). In febrile people, *S. pneumoniae* was more frequently
176 detected in children under five years old (15.1%, 19/126), followed by the 5-20 year old age
177 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
189 observed in children under the age of five (4.8%, 6/126; $p=0.001$).

190 *S. aureus* was detected in 4.2% (33/784) of subjects, more precisely in 3.6% (16/438) of
191 febrile people and 4.9% of afebrile people (17/346, $p=0.383$). No statistical difference was
192 observed according to location or age. *S. pyogenes* was only detected in three febrile children
193 under the age of five (Table 3).

194 The prevalence of *R. felis* was 5.9% (46/784) among all individuals, of whom 5.5%
195 were febrile (24/438) and 6.4% were afebrile (24/364, $p=0.603$). Its prevalence in febrile
196 patients was significantly higher in rural areas (10.3%, 12/322) than in urban areas (3.7%,
197 12/322; $p=0.007$). *T. whipplei* was only identified in four febrile children under the age of
198 eight, all of whom lived in an urban area.

199 **3.3. Co-infections**

200 Co-infections were significantly higher in febrile patients (10.3%, 45/438) than in
201 afebrile people (5.5%, 19/346; $p=0.010$). Most of the co-infections involved *Plasmodium*
202 *falciparum* (82.8%, 53/64). In addition, of the 286 patients who tested positive for *P.*
203 *falciparum*, the probability of being febrile was significantly higher in patients co-infected
204 with *S. pneumoniae* (12/12, 100%) than in mono-infected patients (193/274, 70.0%; $p=0.020$)
205 (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.*
214 *pyogenes* and *S. aureus*, one with *S. pyogenes* and *S. enterica* Typhi, and 2 with *T. whipplei*
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).

222 Overall, 66 of the 217 febrile patients (30.4%) who were positive for *Plasmodium* spp.
223 did not received antimalarial drugs (Figure 4). Of these, 56 were infected with *P. falciparum*
224 alone, 2 two with *P. falciparum* and *P. malariae*, 3 with *P. malariae* alone, and 5 with *P.*

225 *ovale* alone. Conversely, 116 (52.5%) of patients without malaria received antimalarial drugs
226 (Figure 4). Of these, 109 received artemether-lumefantrine, one received artesunate-
227 amodiaquine, 3 quinine, 2 sulfadoxine-pyrimethamine, and one received piperaquine.

228

229 **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

250 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.

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

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

270 *R. felis* was already detected in one *Ctenocephalides canis* collected from a dog in
271 Bouaké, Côte d'Ivoire [34]. However, to the best of our knowledge, this is the first report of
272 *R. felis* bacteraemia in this country. *R. felis* bacteraemia has already been reported in west
273 Africa (Senegal), central Africa (Gabon), and east Africa (Kenya) mainly in febrile people but
274 also in afebrile people [8,35,36]. In Gabon, in the rural area of Fougamou, its prevalence

275 reached 39.7% (23/58) in febrile children, compared to 5.0% (1/20) in afebrile children in the
276 same area and 1.3% (1/77) in febrile children in the city of Franceville [8]. In our study, no
277 difference was observed between febrile and afebrile people, but the prevalence of *R. felis*
278 bacteraemia was significantly higher in rural areas. Finally, *R. felis* was also detected in
279 Senegal in skin swabs from eschars in acute febrile patients and healthy villagers, but not in
280 those of French urban residents [35,37].

281 To the best of our knowledge, this is also the first report in Côte d'Ivoire of *T. whipplei*,
282 the agent of Whipple's disease [38]. In rural Africa, *T. whipplei* has already been detected in
283 faeces, mainly from young, asymptomatic and diarrhoeic children in Gabon, Ghana and
284 Senegal [39–41]. Acute *T. whipplei* bacteraemia has also been reported in febrile people in
285 rural areas of Senegal, including in young children presenting with a cough, but also as a
286 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.

298 The differences between the retrospective molecular diagnosis and the anti-infectives
299 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
306 choices available to physicians very difficult. Current WHO recommendations for the
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

325 *S. enterica* Typhi as well as possible association between co-infection *S. pneumoniae*/malaria
326 with acute febrile episodes. Vaccines against these bacteria are available, so, vaccination
327 campaigns in sub-Saharan Africa may decrease acute febrile morbidity associated with these
328 preventable microorganisms.
329

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493

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

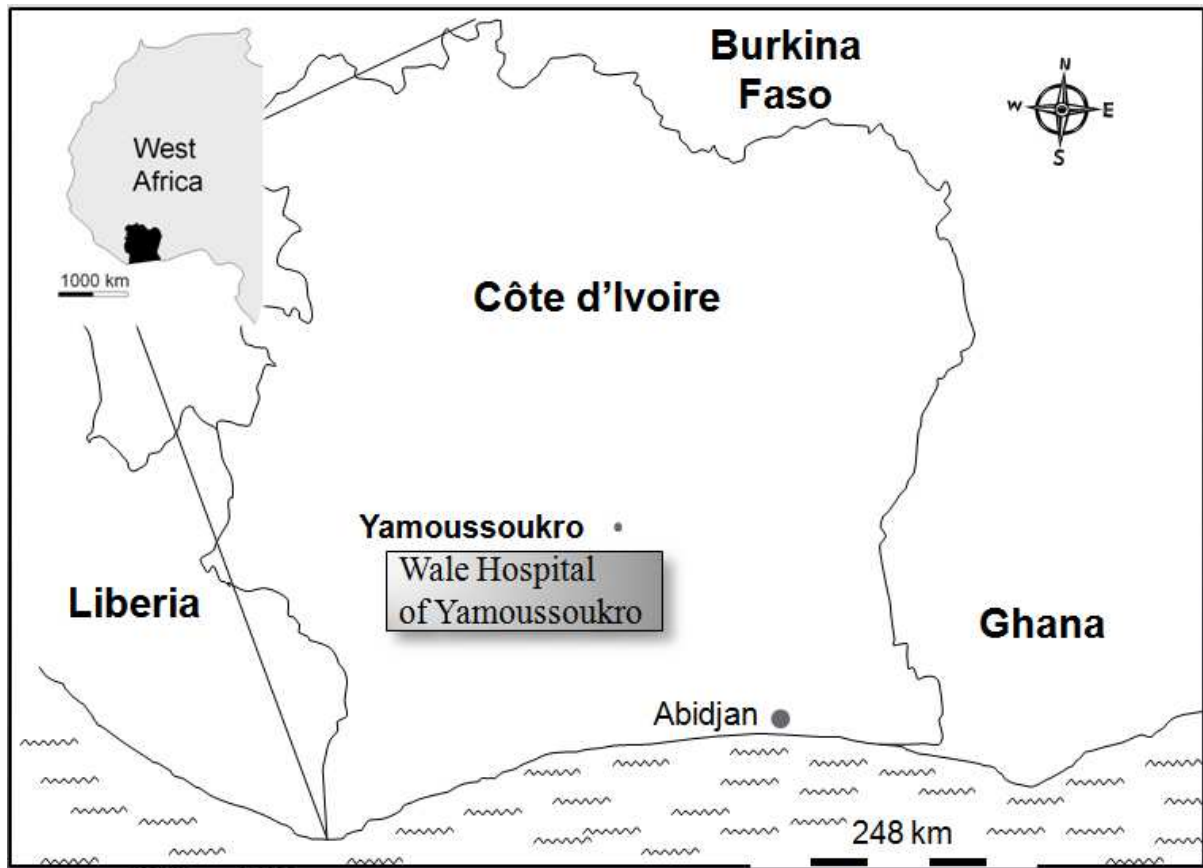


Figure 1. Map of the study site in Côte d'Ivoire.

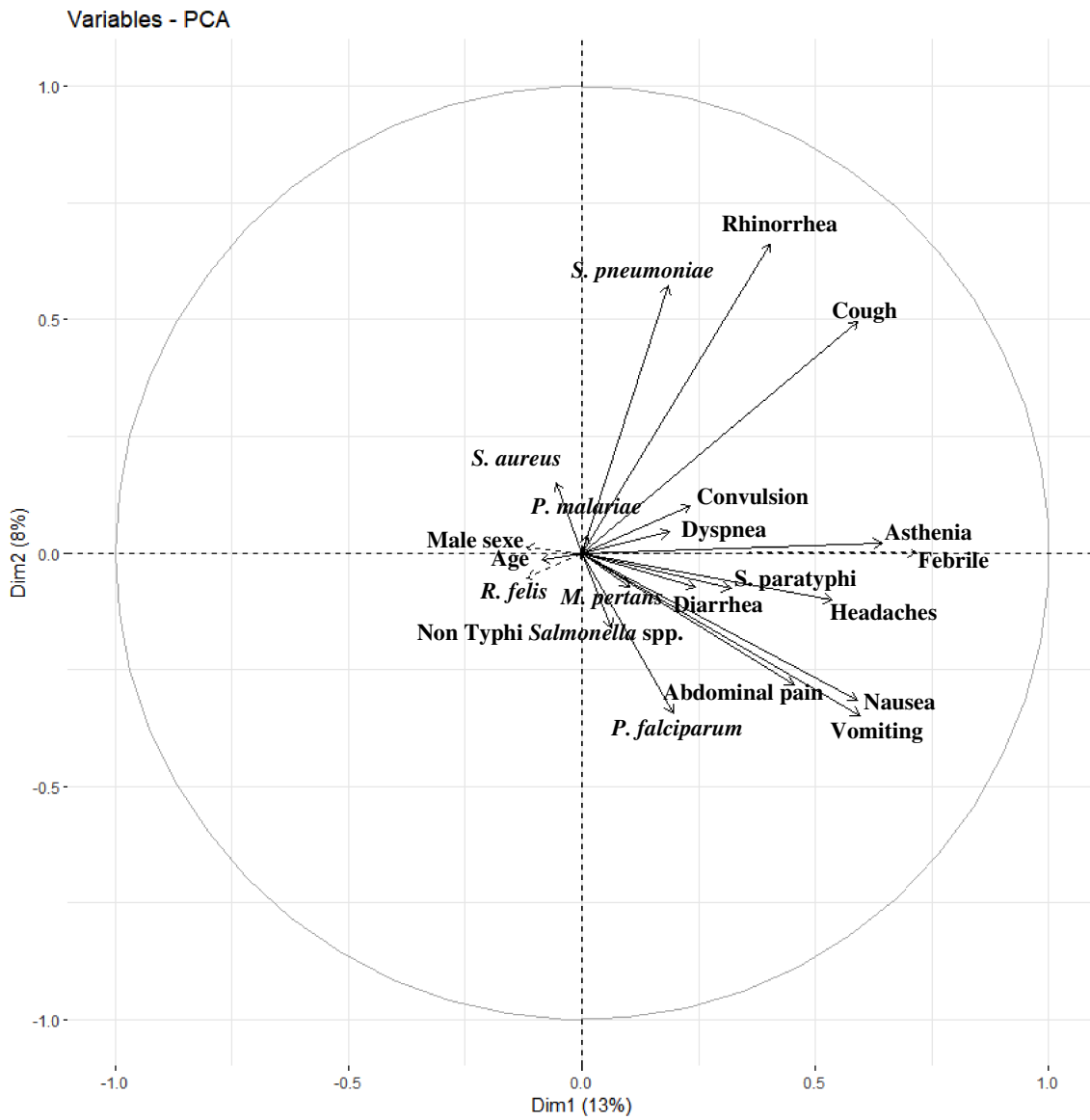


Figure 2. Principal Component Analysis (PCA) of clinical and biological outcomes, demographic characteristics of 348 febrile patients and 346 afebrile people.

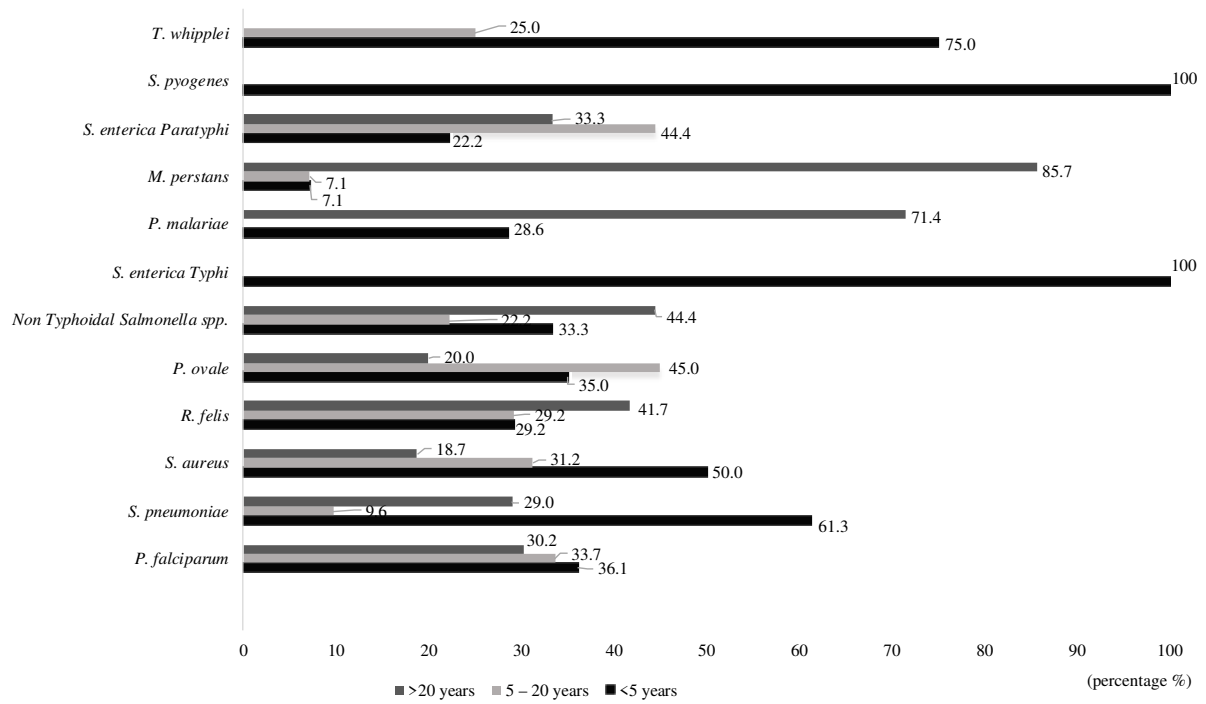


Figure 3. Prevalence of microorganisms detected among the 438 febrile patients according to age.

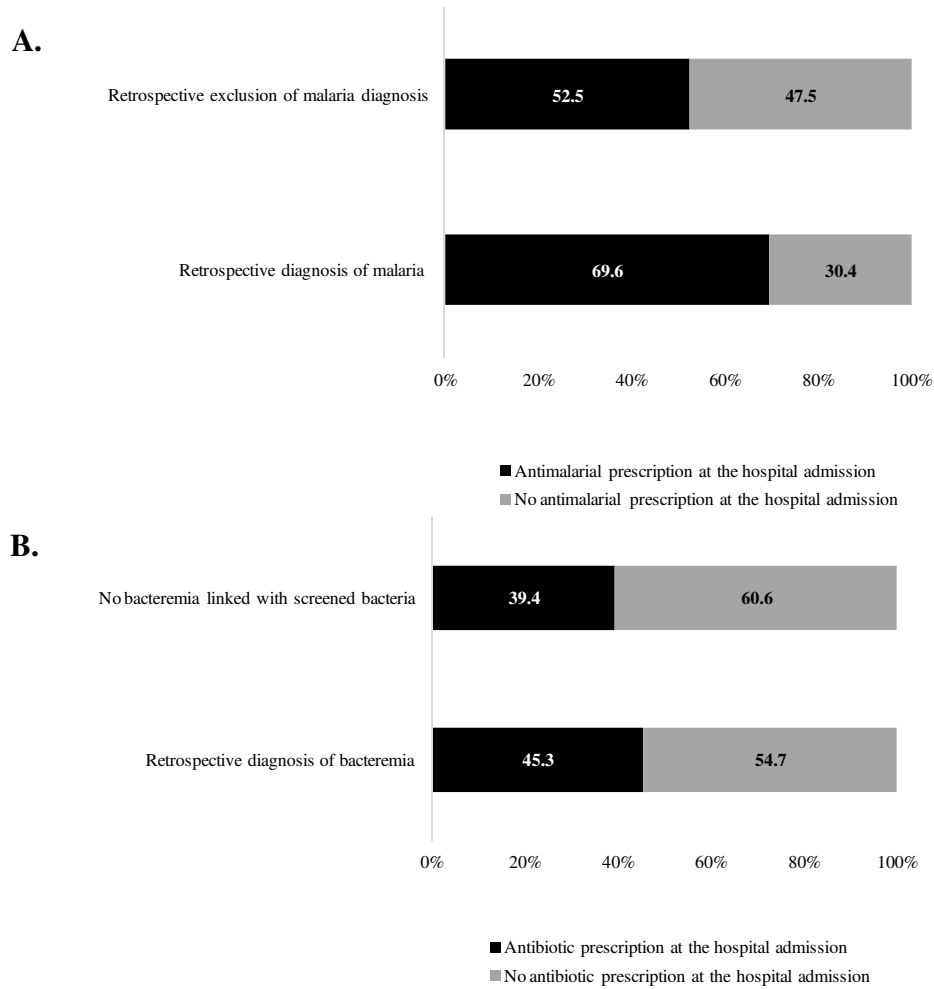


Figure 4. Rate of antimalarial (A) and antibiotic (B) prescriptions upon admission to hospital compared to retrospective molecular diagnostic outcomes.

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.

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 (%)		<i>p. value</i> *
Bacteria			
<i>S. pneumoniae</i>	31 (7.1)	2 (0.6)	<0.001
<i>R. felis</i>	24 (5.5)	22 (6.4)	0.603
Non-typhoidal <i>Salmonella enterica</i>			
<i>S. aureus</i>	16 (3.6)	17 (4.9)	0.383
<i>S. enterica</i> Paratyphi	9 (2.1)	0	0.005
<i>S. enterica</i> Typhi	6 (1.4)	0	0.030
<i>T. whipplei</i>	4 (0.9)	0	0.134
<i>S. pyogenes</i>	3 (0.7)	0	0.259
Parasites			
<i>Plasmodium</i> spp.	217 (49.5)	85 (24.6)	<0.001
<i>P. falciparum</i>	205 (46.8)	81 (23.4)	<0.001
<i>P. ovale</i>	20 (4.6)	0	<0.001
<i>P. malariae</i>	7 (1.6)	8 (2.3)	0.468
<i>Mansonella perstans</i>	13 (3)	11 (3.2)	0.865
No samples were positive for <i>C. burnetii</i> , <i>Borrelia</i> spp., <i>Bartonella</i> spp., and <i>Leptospira</i> spp.			
* Significant <i>p. value</i> (<0.05) are in bold (Chi-square or Fisher's exact tests, 2-sided <i>p. values</i>).			
B.	322 febrile urban inhabitants	116 febrile rural inhabitants	
Microorganisms	Number of positive (%)		<i>p. value</i>
Bacteria			
<i>S. pneumoniae</i>	20 (6.2)	11 (9.5)	0.239
<i>R. felis</i>	12 (3.7)	12 (10.3)	0.007
Non-typhoidal <i>Salmonella enterica</i>			
<i>S. aureus</i>	12 (3.7)	6 (5.2)	0.501
<i>S. enterica</i> Paratyphi	7 (2.2)	2 (1.7)	0.770
<i>S. enterica</i> Typhi	2 (0.6)	4 (3.5)	0.045
<i>T. whipplei</i>	4 (1.2)	0	0.500
<i>S. pyogenes</i>	2 (0.6)	1 (0.9)	1.000
Parasites			
<i>Plasmodium</i> spp.	145 (45.0)	72 (62.1)	<0.001
<i>P. falciparum</i>	135 (41.9)	70 (60.3)	0.007
<i>P. ovale</i>	15 (4.7)	5 (4.3)	0.878
<i>P. malariae</i>	3 (0.9)	4 (3.4)	0.064
<i>M. perstans</i>	6 (1.0)	7 (6.0)	0.023

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

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

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

	438 febrile patients 346 afebrile people		<i>p</i> . value
	Number of positive (%)		
Co-infections	45 (10.3%)	19 (5.5%)	0.01
Triple infections	8 (1.8%)	2 (0.6%)	0.100
<i>P. falciparum</i> / <i>M. perstans</i> / Non-typhoidal <i>Salmonella</i>	0	1 (0.3)	0.441
<i>P. falciparum</i> / <i>P. malariae</i> / <i>S. aureus</i>	0	1 (0.3)	0.441
<i>P. falciparum</i> / <i>P. malariae</i> / <i>S. pneumoniae</i>	1 (0.2)	0	0.999
<i>P. falciparum</i> / <i>P. ovale</i> / <i>R. felis</i>	1 (0.2)	0	0.999
<i>P. falciparum</i> / <i>P. ovale</i> / <i>S. aureus</i>	2 (0.5)	0	0.506
<i>P. falciparum</i> / <i>R. felis</i> / <i>S. aureus</i>	1 (0.2)	0	1.000
<i>P. falciparum</i> / <i>S. aureus</i> / <i>S. pneumoniae</i>	2 (0.5)	0	0.506
<i>P. ovale</i> / <i>S. aureus</i> / <i>S. enterica</i> Typhi	1 (0.2)	0	0.999
Double infections	37 (8.5%)	17 (4.9%)	0.05
<i>P. falciparum</i> / <i>M. perstans</i>	2 (0.5)	3 (0.9)	0.659
<i>P. falciparum</i> / <i>R. felis</i>	8 (1.8)	6 (1.7)	0.922
<i>P. falciparum</i> / <i>S. aureus</i>	10 (2.3)	3 (0.9)	0.162
<i>P. falciparum</i> / Non-typhoidal <i>Salmonella</i>	10 (2.3)	2 (0.6)	0.077
Non-typhoidal <i>Salmonella</i> / <i>M. perstans</i>	2 (0.5)	1 (0.3)	0.999
Non-typhoidal <i>Salmonella</i> / <i>T. whipplei</i>	2 (0.5)	0	0.999
<i>R. felis</i> / <i>S. aureus</i>	1 (0.2)	1 (0.3)	0.999
<i>R. felis</i> / Non-typhoidal <i>Salmonella</i>	1 (0.2)	0	0.999
<i>R. felis</i> / <i>S. enterica</i> Typhi	1 (0.2)	0	0.999
<i>R. felis</i> / <i>M. perstans</i>	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
<i>S. pneumoniae</i>	Amoxicillin	8
	Amoxicillin and clavulanic acid	1
	Amoxicillin / Metronidazole	1
	Cefadroxil	2
	Rifamycin / Enrofloxacin/ Cefpodoxime	1
	Cefadroxil / Ethambutol	1
<i>R. felis</i>	Amoxicillin	2
	Cefadroxil	1
	Neomycin / Bacitracin	1
	Metronidazole	1
	Penicillin	1
<i>S. enterica</i> Typhi	Amoxicillin and clavulanic acid	3
	Cefadroxil	2
	Nifuroxazide	1
	Amoxicillin and clavulanic acid	1
Non-typhoidal <i>Salmonella</i> spp.	Amoxicillin	4
	Azythromycin	1
	Amoxicillin and clavulanic acid	3
<i>S. enterica</i> Paratyhi / <i>S. aureus</i>	Amoxicillin	1
	Amoxicillin and clavulanic acid	1
<i>S. aureus</i> / <i>S. pneumoniae</i>	Amoxicillin	1
	Cefadroxil	1
<i>S. enterica</i> Paratyhi	Amoxicillin	1
<i>S. aureus</i>	Flucloxacillin	1
<i>R. felis</i> / <i>S. enterica</i> Typhi	Cefadroxil	1
<i>T. whipplei</i> / non-typhoidal <i>Salmonella</i> spp.	Amoxicillin and clavulanic acid	1