## Febrile diseases in young children in Burkina Faso

*Etiologies and the value of rapid diagnostic test in primary healthcare settings*

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**Francois Kiemde**

Colofon

FEBRILE DISEASES IN YOUNG CHILDREN IN BURKINA FASO: ETIOLOGIES AND THE VALUE OF RAPID DIAGNOSTIC TESTS IN PRIMARY HEALTHCARE SETTINGS

# FEBRILE DISEASES IN YOUNG CHILDREN IN BURKINA FASO:

# ETIOLOGIES AND THE VALUE OF RAPID DIAGNOSTIC TESTS IN PRIMARY HEALTHCARE SETTINGS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op vrijdag 21 juni 2019, te 12.00 uur

door Francois Kiemde

geboren te Adjame

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Faculteit der Geneeskunde

# *Dedication*

To the memories of Ouanga Azara and Kafando N. Dominique;

To my father, step-mothers, brothers and sisters;

To Arielle, Anael, Ange and Gwladys;

To my aunts Rasmata Leontine and Mariam;

To my family in-law.

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# *Chapter 1*

*General introduction and outline of the thesis*

***Background***

In Burkina Faso, like many other sub-Saharan African (SSA) countries, fever remains the first reason for medical consultation in children under 5 years of age (1). Traditionally, in areas without laboratory facilities, fever was presumptively managed. For example, it was common practice in malaria endemic settings to treat every febrile episode as malaria without determining the actual cause of fever (2) (3). This practice has changed due to the worldwide adoption of the recommendation of the World Health Organization (WHO) to diagnose every presumptive malaria case prior to the administration of antimalarial treatment (4) (5) (6). This "test and treat" strategy has been made possible by the introduction of rapid diagnostic tests (RDTs) for malaria. These RDTs are easy to perform and provide a diagnostic result within 30 minutes (7).

This approach has led to a significant reduction in antimalarial prescriptions based on presumption. At the same time, however, healthcare workers face another challenge: the adequate management of malaria negative cases following an RDT. Given the lack of appropriate diagnostic tools for the diagnosis of non-malaria infections, antibiotics are prescribed by community health workers or nurses to treat the possible cause of fever, without knowing the actual etiology. The efficacy of these antibiotics is, however, not infinite, and antibiotic resistance threatens the effectiveness of the successful treatment of infections and is a public health concern with national and global dimensions (8). Due to the diagnostic constraints of RDTs, inappropriate treatment can also still occur in patients tested for malaria. Patients with a false negative malaria RDT are often treated with antibiotics regardless of the etiology of their infection, whilst all false positive malaria RDT cases receive unnecessary antimalarial treatment. Consequently, the inappropriate use of antimalarials, which was general practice before the introduction of malaria RDTs, has now been replaced by an overuse of antibiotics in order to not miss potential treatable bacterial infections.

The aim of this thesis is to address the problem of the diagnosis and management of febrile diseases in young children living in rural settings in Burkina Faso. Our aim is to provide more insight into the etiology of febrile disease in the study region, to determine the performance of current diagnostic practices and their effect on drug prescriptions, and to propose some diagnostic alternatives to improve the diagnosis and management of febrile diseases.

***Fever in children: thermal regulation of the human body and clinical evidence of infection***

Many patients present to healthcare facilities with the complaint of "fever." However, it is not always possible to establish objectively whether a child has fever. There are many challenges in measuring "true" body temperature in a clinical setting, which include, amongst others, variation of the body temperature of children according to level of activity, meals, time of day, environmental conditions, improper positioning of thermometers, and hurried measurements (1) (9) (10) (11) (12) (13) (14) (15) (16) (17). These factors are difficult – if not impossible – to control for.

Body temperature is an important vital sign providing information about the health status of a patient. In children under 5 years of age, fever remains the fundamental clinical indicator of infectious diseases. Nowadays, it is known that the regulation of body temperature is a complex adaptive response of the host to invasive pathogens (18). Body temperature is regulated by the coordinated actions of various independent thermo-effector loops, each with its own function (19) (20) (21). It is also important to note that a universally accepted definition of "fever" remains elusive (22) (23). The WHO expert vaccines study group defines "fever" as an axillary temperature of ≥37.5°C (9). However, the Brighton Collaboration Fever Working Group defines "fever" as a body temperature of ≥38°C, irrespective of how this has been measured (23).

The initiation, manifestation and control of a fever episode depends on pyrogens and cryogens, which respectively lead to fever (directly or indirectly) or prevent excessive temperature elevation (24). The height and duration of fever is determined by the balance in the interaction between these pyrogens and cryogens (25). Pyrogens are either exogenous (produced by micro-organisms) or endogenous (produced by the host, mainly pyrogenic cytokines) (26). Cryogens, on the other hand, act as inhibitors to the production of pyrogenic cytokines, and includes anti-inflammatory cytokines, hormones and many neuroendocrine products, and cytochrome P450 (26) (27) (28). The induction of fever in endothermal animals has an important metabolic cost. For example, a 1ºC elevation in body temperature requires a 10–12.5% increase in metabolic rate (29). There is evidence that the increase in body temperature during a fever episode is associated with improved survival and the resolution of many infections. This is the case, for example, in humans infected with the influenza virus, where the treatment of fever with antipyretics could negatively affect patient outcomes (30) (31) (32). However, fever is not always beneficial for patients and could have deleterious effects on outcomes by prolonging illness (33). Based on the etiologies of febrile diseases in SSA, it is evident that exogenous

pyrogens remain the main cause of fever in children. However, the etiologies of fever episodes are various (parasites, bacteria, viruses), making management challenging in areas without laboratory facilities.

In peripheral heath facilities in SSA, such as those where the research for this thesis took place, fever episodes are usually associated with an infectious disease, and are subsequently treated as such, even when the child has another underlying medical problem (34). A major reason for this is the fact that appropriate diagnostic tools, such as point-of-care (POC) tests to differentiate treatable and untreatable etiologies of fever, with the exception of malaria, are not available (35) (36). Unexplained fevers are often treated according to algorithms that are only based on non-specific signs and symptoms, not able to distinguish between different pathogens, which may lead, for example, to inappropriate prescriptions of antimicrobials (37) (38) (39) (40) (41).

***Implementation of malaria RDTs in endemic areas***

For many years, infectious diseases in low and middle income countries (LMICs) have been managed presumptively on the basis of clinical signs and symptoms (42) (43). Nowadays, in primary healthcare settings in many LMICs, a RDT for malaria infection has been added to the diagnostic set-up. These *Plasmodium* antigen(s)-based RDTs are considered to be a reliable tool for the (partial) diagnosis and management of malaria infections in rural settings (4) (5) (6). They have the following advantages: low cost, fast, easy to read, and can be performed outside a conventional laboratory near the patient (POC). There were 53 RDTs products from 27 companies available for malaria diagnosis during the WHO-FIND testing round 6 based on the detection of the following antigens: *Plasmodium falciparum* histidine-rich protein-2 (*Pf*HRP2), *Plasmodium* lactate dehydrogenase (*p*LDH) and aldolase (44). The most commonly used in SSA is the RDT based on *Pf*HRP2, which is specific for *Plasmodium falciparum* only (45) (46). In addition, the *p*LDH detecting RDT, which can detect all human *Plasmodium* species, is also authorized by the WHO-FIND program, as well as the two-step RDT which combines both antigens (*Pf*HRP2 and *p*LDH) (47). Nowadays, many community-based initiatives for (early) diagnosis of malaria infection have been made possible by these RDTs (48) (49) (50). This has made malaria RDTs an indispensable tool in the diagnosis of fever etiology.

As the role of malaria RDTs in primary healthcare settings has now been demonstrated, their diagnostic performance – compared to microscopy as the gold standard – is continuously being

monitored under field conditions, in particular in response to a declining intensity of malaria transmission (51) (52) (53) (54) (55). These studies reveal that the diagnostic performance of a malaria RDT may turn out to be below expectations. The principle of the RDT is based on the detection (with monoclonal antibodies) biomarkers associated with the pathogens (56) (57). The bioavailability and the time of metabolism of these markers after successful treatment could, however, negatively impact diagnostic accuracy. For example, the persistence of the HRP2 antigen in blood up to 4 weeks after successful antimalarial treatment contributes to the reduced specificity of the *Pf*HRP2 test (51) (52) (53) (54) (55) (58) (59). Consequently, the HRP2-based test may overestimate the presence of malaria infections. In contrast, unlike *Pf*HRP2, *p*LDH is metabolized within one week, but RDTs based on the detection of this antigen are less sensitive compared to *Pf*HRP2-based tests (detection limit ≥200 parasites/µl) (60). The low sensitivity of *p*LDH-based RDTs may lead to false negative tests results, specially in children with a low parasitemia.

***Challenges for adequate diagnosis of febrile children in sub-Saharan Africa***

In a context of rationalization of antimicrobial prescriptions in LMICs, knowledge of other causes of fever, as well as information on the accuracy of available diagnostic tools, are necessary in the management of fever episodes. The WHO recommendations for malaria management ("test and treat") are having a major impact on the management of fever episodes in general. Indeed, malaria RDTs have become indispensable in the management of febrile episodes. Yet if health workers adhere to the WHO guidelines, they face two challenges: (i) the diagnosis of the true cause of fever (i.e. non-malaria infections) when a malaria RDT is negative, which could be parasitic (other than malaria), viral or bacterial, and for which practical diagnostic tools are not (yet) readily available (61); and (ii) the reliability of the results of malaria RDTs, due to either their low specificity (*Pf*HRP2) or low sensitivity (*p*LDH) (51) (52) (53) (54) (55) (58) (59) (60). In response, many healthcare workers, in order not to miss potentially treatable infections, prescribe a wide range of antibiotics, with a serious risk of contributing to the spread of antibiotic resistance in SSA countries (62) (63) (64) (65).

In Burkina Faso, the etiologies of fever episodes are various (parasites, bacteria, viruses), and depend on age, host and socio-economic characteristics (66). However, very little information is available about the etiologies of fever episodes in our study area (Nanoro). Furthermore, apart from the false positive and negative issue described above, the accuracy of the malaria RDTs in Burkina Faso is often questioned, because factors such as operator errors, training of healthcare works, transport and storage of test may affect its accuracy. Moreover, the effect of

malaria RDTs on the management of other causes of fever has rarely been evaluated. Based on the limits of malaria RDTs, the etiologies of fever episodes and the value of RDTs in the management of these etiologies deserve to be assessed after the roll-out of malaria RDTs in rural settings.

***Aim and outline of the thesis***

This thesis aims to provide a better understanding of the possible cause(s) of fever in children under 5 years of age in the study area of Nanoro, and to determine the value of current diagnostic practices and their effect on antimicrobial prescriptions. Furthermore, it aims to assess alternative approaches to the diagnosis of febrile diseases. The thesis is subdivided into three parts. In the first part, a literature review of possible etiologies of non-malaria fever in febrile children under 5 years of age in SSA is presented (**Chapter 2).** Subsequently, the treatable causes of fever among young children in the research area are determined (**Chapter 3**). In the second part, the diagnostic accuracy of malaria RDTs is evaluated (**Chapters 4** and **5**) and the effect of the use of malaria RDTs on the prescription practices of antimicrobials is described (**Chapter 6**). In the third part, two approaches to improving the diagnosis and management of febrile diseases are presented. In this part, it is assessed whether clinical signs and symptoms, combined with some basic hematology data, can be used to predict the presence of bacterial infections in children under 5 years (**Chapter 7**). Furthermore, in **Chapter 8**, two algorithms for the sequential reading/interpretation of febrile patients with a malaria RDTs that detect two different targets of *Plasmodium* species are described. Finally, the findings of the thesis are discussed and concluded in **Chapter 9**.

***Study area***

The study was conducted in and around Nanoro, a rural area located in the centre-west region of Burkina Faso, around 100 km from Ouagadougou, the capital city. The climate is Sudano-Sahelian with two seasons: the rainy season (June-October, with temperatures ranging between 27–30°C) and the dry season (November-May, with temperatures varying between 17–43°C) (67). The current population is about 65,500 people (Source: HDSS, 2017 estimation). Almost 90% of the population depends on agriculture (68). Peripheral health facilities located in the villages are the first point of medical contact between patients and health workers. The latter follow the Burkina Faso guidelines for disease management (69). Severely ill patients are referred to the Saint Camille district hospital in Nanoro, where healthcare is provided by medical doctors. The under 5 mortality rate in Nanoro region is 31 deaths/1,000 live births

(70). Malaria transmission is hyper-endemic and occurs between June and November. Vaccination against *Haemophilus influenza* type b was introduced in an extended program of immunization (EPI) in 2006. In 2013, vaccines against pneumococcal disease and rotavirus were also added to the package.


**Figure 1: Nanoro, Burkina Faso.**

The research described in this thesis was mainly conducted at the laboratory of the Clinical Research Unit of Nanoro (CRUN, Burkina Faso) and the laboratory of Experimental Parasitology at the Department of Medical Microbiology of the Academic Medical Centre at the University of Amsterdam (The Netherlands). Data and clinical specimen were collected in four health facilities (Nanoro, Godo, Nazoanga and Seguedin) and the referral hospital Saint Camille of Nanoro (Figure 1) with a fully functioning clinical laboratory (new equipment) started in 2009 (68). Malaria microscopy is performed by an expert laboratory technician at CRUN submitted to an external quality control program (National Institute for Communicable Diseases/World Health Organization: NICD/WHO) and only these also certified expert microscopists are allowed to read malaria slides. The microbiology laboratory of CRUN is submitted to internal and external quality control (NICD) according to a standard auditing protocol. For the biochemistry and hematology laboratory, equipment is subjected to a periodic maintenance program and validation with a control sample provided by the manufacturers. The clinical and research laboratories of CRUN are in a process of accreditation of ISO 15189 and 17025 respectively.

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# Chapter 2

## *The aetiologies of non-malaria febrile episode in children under-5 years in sub-Saharan Africa: a systematic review*

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**Abstract:**

**Objectives**: To provide an overview of the most frequent aetiologies found in febrile episodes of children under 5 years from sub-Saharan Africa.

**Methods**: MEDLINE and EMBASE were searched for publications in English and French on non-malaria fever episodes in African children under-5 years of age, which were published between January 1990 and July 2015. Case reports and conference abstracts were excluded.

**Results:** In total, 3851 titles and abstracts were reviewed, and 153 were selected for full screening of which 18 were included in the present review. Bloodstream infection (BSI) was most commonly investigated (nine of 18) followed by urinary tract infection (UTI) (four of 18) and respiratory tract infection (RTI) (two of 18). Few studies investigated BSI and UTI in the same children (two of 18), or BSI and gastrointestinal infection (GII) (one of 18). As for BSI, the most frequently isolated bacteria were *E. coli* (four of 12), *Streptococcus pneumonia* (four of 12), *Salmonella spp* (three of 12) and *Staphylococcus aureus* (two of 12) with a positive identification rate of 19.7–33.3%, 5.2–27.6%, 11.7–65.4% and 23.5–42.0%, respectively. As for UTI, the main bacteria isolated were *E. coli* (six of six) and *Klebsiella spp* (six of six) with a positive rate of 20.0–72.3% and 10.0–28.5%, respectively. No bacterium was isolated in RTI group, but *Human influenzae* A and B were frequently found, with the highest positive identification rate in Tanzania (75.3%). Dengue virus (two of 12) was the most frequently reported viral infection with a positive identification rate of 16.7–30.8%. Finally, only rotavirus/adenovirus (69.2% positive identification rate) was found in GII and no bacterium was isolated in this group.

**Conclusions**: The high prevalence of treatable causes of non-malaria fever episodes requires a proper diagnosis of the origin of fever followed by an appropriate treatment, thereby reducing the under-5 mortality in sub-Saharan Africa and preventing the overprescription of antibiotics and thus circumventing the rise of antibiotic resistance.

**Keywords:** malaria, fever, sub-Saharan Africa, aetiology, children.

**Introduction**

Fever is a very common clinical symptom in children and one of the leading causes for medical consultation in this age group (1). In sub-Saharan Africa, febrile episodes remain the dominant indication for diseases in children under the age of five and represents 6–30% of all practice visits (2) (3) (4) (5). Globally, over six million children die before reaching their fifth birthday (6). Half of this mortality is caused by infectious diseases such as malaria, and bacterial or viral infections (6) (7).

For many years, malaria was one of the commonest causes of death and it was routine practice to treat every febrile illness as malaria (8) (9). Given the high prevalence rate of malaria parasites in children living in endemic areas, the contribution of malaria to the cause of fever may have been overestimated. Fortunately, over the last decade, malaria incidence, morbidity and mortality have fallen in many parts of the world (10). This positive development has been attributed to the global efforts to prevent, to accurately diagnose and to efficiently treat a malaria infection at an early stage (11). Indeed, investigations including febrile episodes in under-5 children have indicated that bacterial and viral aetiologies may now have become the leading causes of fever in this vulnerable age group (12) (13) (14) (15) (16) (17). The rising incidence of these so-called non-malaria fevers has created a diagnostic and treatment dilemma for the clinician and health worker in the field: "what are the possible causes of these fever episodes in children under-5 years of year and are they treatable?"

To better understand the possible aetiologies of fever in children consulting medical advice, we conducted a systematic literature review to assess the most frequent aetiologies of febrile episode in under-5 children in sub-Saharan Africa from January 1990 to August 2015.

**Method**

**Search strategy**

We searched the literature to identify studies reporting data on aetiologies of non-malaria fever episodes in children under-5 years of age in sub-Saharan Africa. MEDLINE was searched on 10 July 2015 (covering the period: January 1990–July 2015) and EMBASE on 04 September 2015 (covering the same period). We searched these databases using the Ovid interface with a combination of MeSH/ENTREE terms and free text words (see Appendix). We combined terms for children, fever and sub-Saharan countries. A complementary search was carried out on 6$^{th}$ of September 2015 in African Index Medicus (covering the period: 1990–2015) by also

using MeSH term and free word text (see Appendix for search strategy). We checked reference lists of all relevant primary studies to look for additional relevant publications.

**Selection criteria**

All studies meeting the inclusion criteria were included. These were studies on aetiologies of fever episodes with laboratory data on pathogens detected or isolated except malaria, enrolment of children under-5 years or containing separate data for this age group, studies conducted in sub-Saharan Africa, data collected between January 1990 and July 2015, and French or English as publication language. The exclusion criteria were literature reviews, study including children under-5 years but without separate data for this age group, case reports of fever diseases, conference abstracts, and papers without source of publication. Titles and abstracts were screened, and eligible studies were downloaded to enable full assessment.

When the age criteria were not strictly clear to the upper limit (5 years or 60 months of age), we checked the descriptive data on age of each paper concerned to confirm that the majority of participants are under-5 years. When more than one publication was identified for the same study, we designated the publication with the most comprehensive data as the main publication. When publications referred included sites in multiple countries including sub-Saharan Africa, these publications were treated by country if there were separate data for these countries. When publications concerned non-malaria febrile episodes coinvestigated with malaria, the data on non-malaria fever were treated without taking malaria data into account. As study designs varied, we took care to check the age criteria and the fever status of children included in these studies. In case of uncertainty on eligibility of a study, the paper was read by a 2$^{nd}$ reader and discussed to reach consensus.

**Results**

**Literature review**

In total, 3865 titles and abstracts were reviewed, of which 3712 were excluded after the first round of screening because they did not meet the eligibility criteria. Subsequently, the full text of 153 publications was reviewed, and 18 studies were included in the final analysis. The reasons of exclusion of 135 articles after reading the full texts are summarized in the PRISMA flow chart (Figure 1). One study on the aetiologies of non-malaria fever was published twice (18) (19) and another study on this subject three times (20) (21) (22). Only studies with sufficient data on the aetiologies were considered for this literature review.

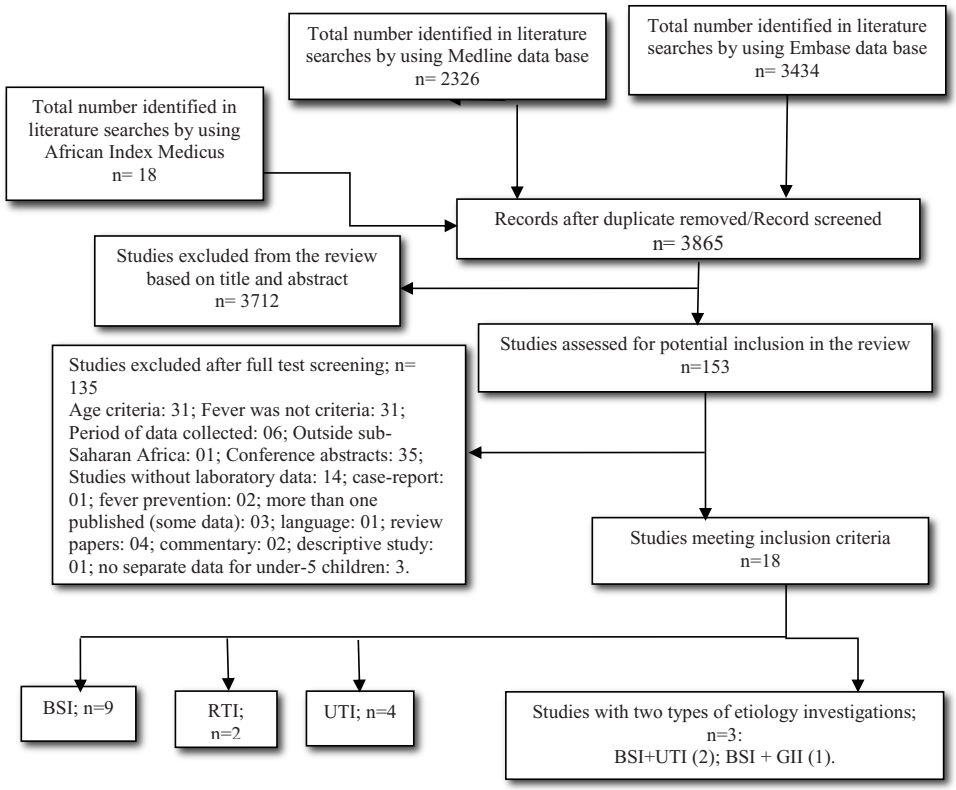


**Figure 1: PRISMA flowchart of study selection, retrieval and inclusion**
BSI=Bloodstream Infection; GII: Gastro-intestinal infection; RTI=Respiratory tract infection; UTI=Urinary tract infection

The studies regarding the aetiology of fever in children under-5 years in sub-Saharan Africa that could be included had a non-homogenous geographic distribution. The 18 studies included in our final analysis (see Table 1) were conducted in eight African countries but two-thirds from sites located in Nigeria (six of 18) and Tanzania (seven of 18). The study period of some studies included did not cover the whole year leaving a gap in the distribution of the aetiologies of fever episodes in the other parts of the year.

The articles included in the current review can be classified in four groups according to their scope. We identified an unequal distribution of data by type of infection. Bloodstream infections (BSI) were investigated in nine of the 18 studies (Figure 1 and Table 1); urinary tract infections (UTI) in four and respiratory tract infection (RTI) in two as the possible cause of the fever. Two studies investigated BSI in combination with both UTI and one in combination with gastrointestinal infection (GII).

**Table 1: Summery of study characteristics of aetiologies non-malaria fever episode in under-5 living in sub-Saharan Africa.**

| Study | Country | ST | Period | Age (months) | Sample size* | Major criteria of studies | Infection | Performed test | Prevalence (%) |
|---|---|---|---|---|---|---|---|---|---|
| Mahende and *al.* (18) | Tanzania | PS | January-October 2013 | 2-59 | 867 | Age: 2-59 months, Temp.: ≥37.5°C | BSI, UTI and malaria | Blood culture, urine culture and microscopy | BSI=3.2; UTI=17.7 |
| Lundgren and *al.* (27) | Tanzania | PS | January 2006-March 2009 | 0-60 | 464 | Age: 0-60 months, Temp.: ≥37.5°C | BSI and malaria | PCR (BSI and malaria), microscopy for malaria | 8.1 |
| Chistopher and *al.* (28) | Tanzania | PS | September 2011-February 2012 | 2-60 | 317 | Age: 2-60 months, Temp.: ≥37.5°C | BSI | Blood culture | 6.6 |
| Walsh and *al.* (29) | Malawi | PS | September 1996-August 1997 | 0-60 | 1847 | Age 0-60 months Temp.: >38°C | BSI | Blood culture | 17.2 |
| Okunola and *al.* (23) | Nigeria | PS | June-August 2006 | 0-60 | 300 | Age: 0-60 months Temp.: fever | UTI and malaria | Urine culture and microscopy | 9.0 |
| Chipwaza and *al.*** (24) | Tanzania | PS | March-May; August-October 2013 | 24-59 | 364 | Age: 2-15 years Temp.: ≥37.5°C | BSI, malaria and GII | ELISA (Dengue, Chikungunya and Rota/Adenovirus), PCR (Dengue) | |
| Thompson and *al.* (34) | Kenya | RS | July 2008-June 2011 | 0-59 | 1917 | Age: under-5 Fever: >38°C | RTI and malaria | RT-PCR (RTI), microscopy (malaria) | 8.2 |
| Mtove and *al.* (30) | Tanzania | PS | March 2008-February 2009 | 2-60 | 4052 | Age: 2 months-14 years Temp.: ≥37.5°C | BSI and malaria | Blood culture, microscopy | 10 |
| Ayoola and *al.* (20) | Nigeria | PS | June-November 1998 | 1-12 | 102 | Age: 1-12 months Temp.: ≥38°C | BSI | Blood culture | 38.2 |
| Musa and *al.* (35) | Nigeria | PS | April-September 1999 | 1-60 | 300 | Age: 1-6O months Temp.: ≥38°C | UTI | Urine culture | 9 |
| Msaki and *al.* (25) | Tanzania | PS | February-June 2011 | 2-60 | 231 | Age: 2-60 months Temp.: ≥37.5°C | BSI, UTI and malaria | Blood culture, urine culture and microscopy | BSI: 7.4 UTI: 20.3 |
| Tarnagda and *al.* (26) | Burkina Faso | PS | July 2010-May 2012 | 1-60 | 398 | Age: 1 month-83 years; Temp.: ≥38°C | RTI | RT-PCR | 2.61 |
| Thriemer and *al.* (36) | Tanzania | PS | March 2009-Decembre 2010 | 2-59 | 3105 | Age: 2 months-15 years Temp.: ≥37.5°C | BSI and malaria | Blood culture and microscopy | 5.55 |
| Rabasa and *al.* (37) | Nigeria | PS | November 2004-October 2005 | 1-60 | 145 | Age: 1-60 months Temp.: ≥37.5°C | UTI | Urine culture | 13.7 |
| Ibeneme and *al.* (31) | Nigeria | PS | February-April 2010 | 1-59 | 200 | Age: 1-59 months Temp.: ≥37.6°C | UTI | Urine culture | 11 |
| Adedoyo and *al.* (38) | Nigeria | PS | | 0-59 | 130 | Age: 0-59 months Temp.: persistent fever | BSI | ELISA test (Dengue virus) | 30.8 |
| Kibuuka and *al.* (32) | Uganda | PS | May-August 2012 | 0-59 | 250 | Age: 0-59 months, Temp.: >37.5°C | BSI | Blood culture | 19.5 |
| Dahamane and *al.* (33) | Sierra Leone | PS | April 2011-February 2012 | 0-60 | 62 | Age: >15 years Temp.: >38°C | BSI | ELISA test (Lassa fever) | 38.7 |

*Dengue: 16.7%; Influenzae virus: 75.3%; Chikungunya virus: 5.4% Rota/Adenovirus: 69.2%.

BSI: Bloodstream infection; ELISA: Enzyme Linked Immunosorbent Assay; GAS: Group A *Streptococcus*; GII = Gastro intestinal infection; ST=Study Type; NTS: Non-Typhoid Salmonella; PS= Prospective Study; RS: Retrospective Study; RTI= Respiratory tract infection; RT-PCR: Real Time-Polymerization Chain Reaction; Temp.: temperature; UTI= Urinary tract infection.

Blood culture for BSI was the most often used method to investigate fever in children under-5 years of age. Polymerization chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) were used in few cases for BSI (one of 12 and three of 12, respectively). Urine culture and PCR were mainly used for UTI and RTI, respectively. Single-step immunochromatographic lateral-flow test was used for rotavirus/adenovirus detection. The bacteria isolated and considered to be the main cause of the febrile illness are presented in Table 2. Only bacteria which constituted at least 10% of all isolated bacteria for each study are reported.

**Aetiologies of fever in children under-5 years**

Bacterial infections were the most investigated causes of febrile episodes in children under-5 years in sub-Saharan Africa. These infections were the dominant aetiology in BSI (nine of 12) and UTI (six of 6). In contrast, viral infections were investigated as possible cause of fever in BSI (three of 12), GII (one of 1) and RTI (two of 2). None of the studies investigated the role of parasites infections in UTI and GII. None of the studies investigated the aetiologies of fever in children under-5 years in sub-Saharan Africa by screening all relevant foci of infection, thus BSI, UTI, RTI and GII together. Patients included in the studies who were selected for our review were outpatients (23) (24) (25) (26), inpatients (20) (27) (28) (29) (30) (31) (32) (33) or the both (18) (34) (35) (36) (37) (38). The mortality rate in the studies that provided this specific information ranged from 1.4 to 22.5% and specifically concerned the inpatient studies (18) (20) (28) (29) (33) (34).

The prevalence of BSI in children under-5 years with fever (≥37.5°C) in sub-Saharan Africa reported in the studies and included in this review was around 10%. Young children under-12 months of age were observed to be at higher risk compared to those over 12 months. High axillary temperature (≥38°C) was found to be predictive for a bacterial infection in BSI. There is a clear difference in UTI between the two countries from where data could be retrieved (around 10% in Nigeria and 20% in Tanzania, respectively). Children aged between 12 and 35 months were observed to be at particular risk of UTI in these two countries. All studies included demonstrated a relation between UTI caused by bacteria and high axillary temperature (temperature ≥40 °C), and also UTI and sex. Female gender was observed to increase the risk of UTI.

The most prevalent bacteria species isolated after blood cultures in studies included were *E. coli* (four of 12), *Streptococcus pneumoniae* (four of 12), *Salmonella spp* (three of 12) and

*Staphylococcus aureus* (two of 12) with positive rates of 19.7–33.3%, 5.2–27.6%, 11.7–65.4% and 23.5–42.0%, respectively. Other bacteria such as *Klepsilla ssp*, *Pseudomonas ssp*, *Proteus ssp*, *Acinetobacter ssp*, *Hemophilus influenzae* and *Enterobacter cloacae* were reported in a few cases. There was heterogeneity on the repartition of BSI bacteria isolated. Bacteria isolated from UTI were predominately *E. coli* (six of 6) and *Klebsiella ssp* (six of 6) with positive rates of 20.0–72.3% and 10.0–28.5%, followed by *Staphylococcus*, *Proteus* and *Pseudomonas* (Table 2). Samples were analysed in district hospitals (18) (27) (28) (30) (35) (36) (37), reference hospitals (32) (38), teaching hospitals (20) (23) (29) (35) or reference laboratories (24) (26) (34).

Viral infections present in blood, nasopharynges and stool were also investigated as possible cause of febrile illness. Dengue virus was the most frequently reported in children under-5 years in the papers that were included in this review. The prevalence observed was higher in Nigeria than Tanzania (30.8% *vs*. 16.7%). In general, dengue virus was more likely to occur in children over-5 years than under-5 years. Chikungunya and Lassa viruses were only once reported to be responsible for fever in children under-5 years (5.4% and 38.7%, respectively). In GII, rotavirus and adenovirus were the only investigated (one of 1) with a prevalence of 69.2%. The prevalence of influenza virus, the only viral species detected in RTI, was at higher rate in Tanzania compared to Kenya and Burkina Faso: 75.3%, 8.2% and 2.6%, respectively. *Influenzae* virus A and B were the most important species reported in RTI.

**Table 2: Summary of major bacteria isolated or detected from paper included in the literature review.**

| | | Bacteria isolated or detected (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study** | **Type of Infection** | ***E. coli*** | ***Sal. typhi*** | **NTS** | ***S. pneu.*** | ***Klebsiella. Spp*** | ***H. influ.*** | ***S. aur.*** | ***K. pneu*** | ***Proteus spp*** | ***Pseudo.*** | **Enteric G-bacilli** | ***Coliform spp*** | ***Strep. fecalis*** |
| Mahende and *al* (18) | BSI: | - | 65.4 | 7.7 | 15.4 | - | - | - | - | - | - | - | - | - |
| | UTI: | 56.1 | - | - | - | - | - | - | 10.6 | - | - | - | - | - |
| Lundgren and *al.* (27) | BSI | - | - | - | 5.2 | - | 3.0 | - | - | - | - | - | - | - |
| Chistopher and *al.* (28) | BSI | 33.3 | - | - | - | - | - | - | 28.5 | - | - | - | - | - |
| Walsh and *al.* (29) | BSI | - | - | 38.4 | 16.2 | - | - | - | - | - | - | 29.4 | - | - |
| Okunola and *al.* (23) | UTI | 29.6 | - | - | - | - | - | 55.6 | 14.8 | - | - | - | - | - |
| Mtove and *al.* (30) | BSI | 19.7 | - | 31.8 | - | - | 15.1 | - | - | - | - | - | - | - |
| Ayoola and *al.* (20) | BSI | 36.0 | - | - | - | 10.0 | - | 33.0 | - | - | - | - | - | - |
| Musa and *al.* (35) | UTI | 58.0 | - | - | - | 23.0 | - | 19.0 | - | - | - | - | - | - |
| Msaki and *al.* (25) | BSI | 23.5 | 11.7 | - | - | - | - | 23.5 | - | - | - | - | - | - |
| | UTI | 72.3 | - | - | - | - | - | - | 21.2 | - | - | - | - | - |
| Thriemer and *al.* (36) | BSI | - | 34.5 | - | 27.6 | - | - | - | - | - | - | - | - | - |
| Rabasa and *al.* (37) | UTI | 20.0 | - | - | - | 15.0 | - | 10.0 | - | 10.0 | - | - | 45.0 | - |
| Ibeneme and *al.* (31) | UTI | 31.8 | - | - | - | 13.6 | - | 22.7 | - | - | - | - | - | 13.6 |
| Kibuuka and *al.* (32) | BSI | - | - | 24.0 | - | - | - | 42.0 | - | - | 11.0 | - | - | - |

BSI= Bloodstream infection; G-: Gram negative; *H. influ.= Hemophilus influenzae*; *K. pneu.= Klebsiella pneumoniae*; NTS= Non typhoid Salmonella; *Pseudo.= Pseudomonas*; *Sal.= Salmonella*; *S. aur.= Staphylococcus aureus*; *S. pneu= Streptococcus pneumoniae*; *Strep.: Streptococcus*; RTI= Respiratory tract infection; UTI= Urinary tract infection.

**Discussion**

The aetiologies reported in this review were mainly from bacterial origin and few were viral. Parasitic infections were not reported. This is in line with the fact that bacterial and/or viral infections are considered to be the most common causes of non-malaria fever episodes in developing countries (12) (13) (14) (16) (17). However, the absence of bacterial aetiology in GII (39) (40) and RTI leave gaps in the understanding of these aetiologies as cause of fever in children under-5 years (12). The potential underestimation of viral infections might be due to the fact that the main diagnostic methods used to investigate viral infections (ELISA and PCR) require adequate technical facilities, which are not readily accessible in many African settings. Therefore, there are considerably more data available on bacterial than viral infections.

Children under-12 months of age are at high risk of BSI. One possible explanation for this increased risk can be the finding that placental malaria is associated with a higher prevalence of non-malaria infections during the first 18 months of life (41). Placental malaria is frequently observed as a consequence of *Plasmodium falciparum* infection during pregnancy in sub-Saharan Africa (42). Children aged 12–36 months were observed to be at risk of UTI. This age group corresponds to the quest of autonomy where children are less close to their parents or guardians and the lack of supervision, hygiene conditions and good sanitation might explain the high risk in this age group. An example of this is the different prevalence of UTI observed in Tanzania (high) and Nigeria (low) which could be explained by the lack of hygiene and sanitation in the rural community reported in Tanzania (18) (25). Fever >38°C was a predictor of bacterial infection (BSI or UTI). This confirms the conclusion of Amanda et *al*. that bacteraemia is an important cause of fever in children (29).

Although outbreaks of dengue and chikungunya have been reported, data on their incidence and prevalence are not available for Africa (43) (44). The true incidence of these viruses cannot be estimated from the few studies included in this review in which dengue virus was reported. Lassa fever virus infects 100 000 people annually and occurs principally in children under-5 years. It is reported in one study included in this review (45). In GII, viral gastroenteritis caused by rota and adenovirus is the main cause of fever (39). Mortality rate data were only available from studies that included inpatients (possibly because it is easy to access this particular information from studies participants after inclusion). Very high mortality rates were found in a study on BSI in children under-12 months of age (14.70%) (20) and a study on Lassa fever (22.58%) (33), which is a dangerous haemorrhagic fever of children under-5 years of age.

The bacterial aetiologies reported in different studies included were not different with those reported in other studies in sub-Saharan Africa, but had a disparity in geographical repartition (12) (14) (15) (46) (47) (48). The range of bacteria isolated shows that there are regional and seasonal variations across sub-Saharan Africa for BSI. The disparity observed in many areas can to some extend be explained by the coexistence of different risk factors between the population under study, such as home crowding, low hygiene standard and high prevalence of other diseases and conditions such as HIV and malnutrition (48). Despite the availability of data for developing countries, there are significant gaps in understanding mortality, including childhood mortality and morbidity in children under-5 years, caused by infectious diseases (49). This can be attributed to several factors. Firstly, fever episodes remain unexplained in peripheral health facilities in sub-Saharan Africa. The main reason is unavailability of point-of-care test to differentiate pathogens causing diseases, except for malaria (50) (51). Secondly, existing estimations are mainly focused on geographical variation and few studies have investigated seasonal variation in hospital mortality and morbidity rates. However, climate factors such as temperature, wind and humidity, play an important role in the epidemiology of infectious diseases, mainly those that are vector borne or waterborne. These childhood diseases are superimposed in sub-Saharan Africa with peaks during certain periods of the year. This is, for example, the case in the spread and development of RTI where wind and cold remain the principal factors of transport and physiopathology, respectively (52) (53) (54). The availability of data in the repartition of infectious disease morbidity and mortality in hospital and health facilities in developing countries could contribute to delivering appropriated health care to children and rationalizing the use of resources.

Two-thirds of studies included were BSI or BSI in combination with other infections. However, it is known that pneumonia is one of the main causes of fever in children under-5 years and many cases of GII like those caused by rotavirus and adenovirus are also associated with fever (55) (56) (57). There is thus a focus on BSI, whereas other types of infections are less well studied. It is therefore important to have a good balance in research objectives in this age group where the diagnosis of fever is a challenge. This will enable to get a better overview of all causes of fever in children under-5 years. So far, the research on the aetiology of febrile illness depends strongly on the design of the studies and on the methodologies used. It is crucial to use the appropriate method that can detect the relevant type of pathogen. Often PCR or ELISA is being used to identify the pathogen that is assumed to be the cause.

However, there is a risk that less prevalent pathogens are being overlooked with these targeting technologies, because a preselection has been already carried out. Therefore, it is also important to perform cultures to identify the less abundant pathogens. Another relevant issue that was not addressed in any of the studies included in the current review is the implementation of quality control (QC) procedures. Obviously, the quality of the laboratory analyses will influence the standards of reporting and has an effect on the identification of the different pathogens causing fever. However, as mentioned in the results section, all studies included were performed in district hospitals or reference hospitals/laboratories and it is assumed that these meet the minimal requirements in terms of infrastructure, equipment and protocols, to properly conduct the identification of the pathogens concerned.

A potential limitation of our work lies in the fact that only studies with a clear definition of fever ("axillary temperature ≥37.5°C") as a mean criterion for inclusion were included in our literature review. Studies with "history of fever" or "fever-like" inclusion criteria were excluded from the current review. This may have led to the exclusion of potentially interesting data. Furthermore, papers or studies investigating specific syndromes, such as pneumonia, without the inclusion criterion "fever", for example the study by Berkley et *al*. in Kilifi on viral aetiology of pneumonia (58), will also not show up in our search. Thus, our inclusion and exclusion criteria could limit the number of papers selected for the review, but 18 included papers are considered to be sufficient numbers to conduct a systematic review. For example, a review focusing on children AND adults included 22 papers (59). Some studies excluded did not have separate data for febrile and non-febrile children (40) (46) (60). The high burden of malaria reported during the last 25 years in the endemic countries could explain the lack of data of non-malaria fever in this age group as most attention was focused towards malaria (11) (61). The financial costs and adequate technical facilities needed to perform such in-depth studies on bacterial and viral infections could also be another reason for the scarcity of data.

The uneven geographic distribution of included studies in this review was an important gap in the understanding of aetiology of fever episode in children under-5 years in Africa. This proves the lack of interest in non-malaria fever *vs*. malaria fever in children under-5 years in some localities of sub-Saharan Africa. The lack of required scientific data in many countries on the aetiologies of fever episodes in children under-5 years does not allow to draw general conclusions. Despite the availability of some data, it is not possible to extrapolate these to other areas because of local sociocultural, environmental factors and lifestyle particularities.

**Conclusion**

Despite the scarcity of data retrieved by our search, it is clear that a considerable number of febrile episode are caused by treatable aetiologies in children under-5 years of age in sub-Saharan Africa. Proper diagnosis and appropriate treatment of these children would save many lives and prevent the abusive prescription of antibiotics which may lead to growing antibiotic resistance.

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**Appendix: Search strategy**

| Ovid MEDLINE(R) In-Process and Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present (dd 10 July-2015) | | |
|---|---|---|
| # | Searches | Results |
| 1 | exp Fever/ or ((increased adj2 Body adj temperature) or febrile or fever or (high adj2 teperature) or hyperthermia or pyrexia or ((elevat* or raised) adj2 temperature)).ti,ab,kf. | 193573 |
| 2 | exp Child/ or exp Child, preschool/ or exp Infant/ or exp Infant, newborn/ or exp Infant, low birth weight/ or exp Infant, small for gestational age/ or exp Infant, very low birth weight/ or exp Infant, postmature/ or exp Infant, premature/ or exp Child, abandoned/ or exp Child, unwanted/ or exp Minors/ or exp Child hospitalized/ or exp Child institutionalized/ or exp Homeless youth/ or exp Disabled children/ or exp Pediatrics/ or (child$ or paediatric$ or pediatric$ or perinat$ or neonat$ or newborn$ or infan$ or bab$ or toddler$ or boy$ or girl$ or kid$1 or schoolage or underage$ or offspring).mp. or (infan$ or child$ or pediatric$ or paediatric$).jw. | 2864913 |
| 3 | 1 and 2 | 49881 |
| 4 | Cape Verde.ti,ab,kw,in,hw. or exp "Africa South of the Sahara"/ or "Africa south of the Sahara".ti,ab,kw,in,hw. or sub-Saharan.ti,ab,kw,in,hw. or subSaharan.ti,ab,kw,in,hw. or Cameroon.ti,ab,kw,in,hw. or "Central African Republic".ti,ab,kw,in,hw. or Chad.ti,ab,kw,in,hw. or Congo.ti,ab,kw,in,hw. or DRC.ti,ab,kw,in,hw. or Equatorial Guinea.ti,ab,kw,in,hw. or Gabon.ti,ab,kw,in,hw. or Burundi.ti,ab,kw,in,hw. or Djibouti.ti,ab,kw,in,hw. or Eritrea.ti,ab,kw,in,hw. or Ethiopia.ti,ab,kw,in,hw. or Kenya.ti,ab,kw,in,hw. or Rwanda.ti,ab,kw,in,hw. or Somalia.ti,ab,kw,in,hw. or Sudan.ti,ab,kw,in,hw. or Tanzania.ti,ab,kw,in,hw. or Uganda.ti,ab,kw,in,hw. or Angola.ti,ab,kw,in,hw. or Botswana.ti,ab,kw,in,hw. or Lesotho.ti,ab,kw,in,hw. or Malawi.ti,ab,kw,in,hw. or Mozambique.ti,ab,kw,in,hw. or Namibia.ti,ab,kw,in,hw. or South Africa.ti,ab,kw,in,hw. or Swaziland.ti,ab,kw,in,hw. or Zambia.ti,ab,kw,in,hw. or Zimbabwe.ti,ab,kw,in,hw. or Benin.ti,ab,kw,in,hw. or "Burkina Faso".ti,ab,kw,in,hw. or "Cape Verde".ti,ab,kw,in,hw. or "Cote d'Ivoire".ti,ab,kw,in,hw. or Gambia.ti,ab,kw,in,hw. or Ghana.ti,ab,kw,in,hw. or (Guinea not guinea pig*).ti,ab,kw,in,hw. or Guinea-Bissau.ti,ab,kw,in,hw. or Equatorial Guinea.ti,ab,kw,in,hw. or Liberia.ti,ab,kw,in,hw. or Mali.ti,ab,kw,in,hw. or Mauritania.ti,ab,kw,in,hw. or Niger.ti,ab,kw,in,hw. or Nigeria.ti,ab,kw,in,hw. or Senegal.ti,ab,kw,in,hw. or "Sierra Leone".ti,ab,kw,in,hw. or Togo.ti,ab,kw,in,hw. or Zaire.ti,ab,kw,in,hw. or Central Africa*.ti,ab,kw,in,hw. or Eastern Africa*.ti,ab,kw,in,hw. or East Africa*.ti,ab,kw,in,hw. or Southern Africa*.ti,ab,kw,in,hw. or Western Africa*.ti,ab,kw,in,hw. or West Africa*.ti,ab,kw,in,hw. or Comoros.ti,ab,kw,in,hw. or Madagascar.ti,ab,kw,in,hw. or Reunion.ti,ab,kw,in,hw. or Seychelles.ti,ab,kw,in,hw. or "Sao Tome and Principe".ti,ab,kw,in,hw. or Mayotte.ti,ab,kw,in,hw. or Mauritius.ti,ab,kw,in,hw. or "Saint Helena".ti,ab,kw,in,hw. or Abyssinia.ti,ab,kw,in,hw. or Rhodesia.ti,ab,kw,in,hw. or Zanzibar.ti,ab,kw,in,hw. or "German East Africa".ti,ab,kw,in,hw. or Africa.ti,ab,kw,in,hw. or "Upper Volta".ti,ab,kw,in,hw. or "Tripolitania and Cyrenaica".ti,ab,kw,in,hw. or Numidia.ti,ab,kw,in,hw. or "Sudanese Republic".ti,ab,kw,in,hw. or "French Equatorial Africa".ti,ab,kw,in,hw. or "German East Africa".ti,ab,kw,in,hw. | 307004 |
| 5 | 3 and 4 | 4100 |
| 6 | exp incidence/ or exp prevalence/ or exp epidemiology/ or incidence.tw. or prevalence.tw. or frequenc$.tw. or occurrence.tw. or exp cross-sectional study/ or health survey/ or (health adj3 survey$).mp. or cross-sectional.mp. or (population adj3 based).mp. | 2189279 |
| 7 | register.mp. | 40855 |
| 8 | exp Registries/ | 63142 |
| 9 | database.mp. | 164260 |
| 10 | surveillance.mp. or exp Public Health Surveillance/ or exp Population Surveillance/ | 156676 |
| 11 | multivariate.mp. or exp Multivariate Analysis/ or exp Retrospective Studies/ | 752492 |
| 12 | exp Medical Records/ | 91985 |
| 13 | Comparative Study/ | 1727134 |
| 14 | (comparative adj stud*).mp. | 1763564 |
| 15 | etiology.mp. | 157286 |
| 16 | exp Cohort Studies/ or cohort.mp. | 1582797 |
| 17 | or/6-16 | 5097317 |
| 18 | 5 and 17 | 2564 |
| 19 | limit 18 to yr="1990 -Current" | 2326 |

| Embase Classic and Embase 1947 to 04 September 2015. | | |
|---|---|---|
| # | Searches | Results |
| 1 | fever/ | 175402 |
| 2 | ((increased adj2 Body adj temperature) or febrile or fever or (high adj2 teperature) or hyperthermia or pyrexia or ((elevat* or raised) adj2 temperature)).ti,ab,kw. | 263853 |
| 3 | 1 or 2 [fever component] | 345333 |
| 4 | child/ | 1507942 |
| 5 | prematurity/ | 86180 |
| 6 | (child$ or paediatric$ or pediatric$ or perinat$ or neonat$ or newborn$ or infan$ or bab$ or toddler$ or boy$ or girl$ or kid$1 or schoolage or underage$ or offspring).mp. | 3370131 |
| 7 | (infan$ or child$ or pediatric$ or paediatric$).jx. | 645867 |
| 8 | or/4-7 [child] | 3475314 |
| 9 | Cape Verde.ti,ab,kw,in,hw. or exp "Africa South of the Sahara"/ or "Africa south of the Sahara".ti,ab,kw,in,hw. or sub-Saharan.ti,ab,kw,in,hw. or subSaharan.ti,ab,kw,in,hw. or Cameroon.ti,ab,kw,in,hw. or "Central African Republic".ti,ab,kw,in,hw. or Chad.ti,ab,kw,in,hw. or Congo.ti,ab,kw,in,hw. or DRC.ti,ab,kw,in,hw. or Equatorial Guinea.ti,ab,kw,in,hw. or Gabon.ti,ab,kw,in,hw. or Burundi.ti,ab,kw,in,hw. or Djibouti.ti,ab,kw,in,hw. or Eritrea.ti,ab,kw,in,hw. or Ethiopia.ti,ab,kw,in,hw. or Kenya.ti,ab,kw,in,hw. or Rwanda.ti,ab,kw,in,hw. or Somalia.ti,ab,kw,in,hw. or Sudan.ti,ab,kw,in,hw. or Tanzania.ti,ab,kw,in,hw. or Uganda.ti,ab,kw,in,hw. or Angola.ti,ab,kw,in,hw. or Botswana.ti,ab,kw,in,hw. or Lesotho.ti,ab,kw,in,hw. or Malawi.ti,ab,kw,in,hw. or Mozambique.ti,ab,kw,in,hw. or Namibia.ti,ab,kw,in,hw. or South Africa.ti,ab,kw,in,hw. or Swaziland.ti,ab,kw,in,hw. or Zambia.ti,ab,kw,in,hw. or Zimbabwe.ti,ab,kw,in,hw. or Benin.ti,ab,kw,in,hw. or "Burkina Faso".ti,ab,kw,in,hw. or "Cape Verde".ti,ab,kw,in,hw. or "Cote d'Ivoire".ti,ab,kw,in,hw. or Gambia.ti,ab,kw,in,hw. or Ghana.ti,ab,kw,in,hw. or (Guinea not guinea pig*).ti,ab,kw,in,hw. or Guinea-Bissau.ti,ab,kw,in,hw. or Equatorial Guinea.ti,ab,kw,in,hw. or Liberia.ti,ab,kw,in,hw. or Mali.ti,ab,kw,in,hw. or Mauritania.ti,ab,kw,in,hw. or Niger.ti,ab,kw,in,hw. or Nigeria.ti,ab,kw,in,hw. or Senegal.ti,ab,kw,in,hw. or "Sierra Leone".ti,ab,kw,in,hw. or Togo.ti,ab,kw,in,hw. or Zaire.ti,ab,kw,in,hw. or Central Africa*.ti,ab,kw,in,hw. or Eastern Africa*.ti,ab,kw,in,hw. or East Africa*.ti,ab,kw,in,hw. or Southern Africa*.ti,ab,kw,in,hw. or Western Africa*.ti,ab,kw,in,hw. or West Africa*.ti,ab,kw,in,hw. or Comoros.ti,ab,kw,in,hw. or Madagascar.ti,ab,kw,in,hw. or Reunion.ti,ab,kw,in,hw. or Seychelles.ti,ab,kw,in,hw. or "Sao Tome and Principe".ti,ab,kw,in,hw. or Mayotte.ti,ab,kw,in,hw. or Mauritius.ti,ab,kw,in,hw. or "Saint Helena".ti,ab,kw,in,hw. or Abyssinia.ti,ab,kw,in,hw. or Rhodesia.ti,ab,kw,in,hw. or Zanzibar.ti,ab,kw,in,hw. or "German East Africa".ti,ab,kw,in,hw. or Africa.ti,ab,kw,in,hw. or "Upper Volta".ti,ab,kw,in,hw. or "Tripolitania and Cyrenaica".ti,ab,kw,in,hw. or Numidia.ti,ab,kw,in,hw. or "Sudanese Republic".ti,ab,kw,in,hw. or "French Equatorial Africa".ti,ab,kw,in,hw. or "German East Africa".ti,ab,kw,in,hw. | 433425 |
| 10 | exp epidemiology/ | 2363419 |
| 11 | (incidence or prevalence or frequenc$ or occurrence).tw. or exp cross-sectional study/ or health survey/ or (health adj3 survey$).mp. or cross-sectional.mp. or (population adj3 based).mp. | 2783685 |
| 12 | register.mp. | 117505 |
| 13 | exp register/ | 80348 |
| 14 | database.mp. | 281051 |
| 15 | surveillance.mp. | 189115 |
| 16 | exp multivariate analysis/ | 295982 |
| 17 | exp retrospective study/ | 424144 |
| 18 | multivariate.mp. | 326895 |
| 19 | exp medical record/ | 159893 |
| 20 | exp comparative study/ | 1097310 |
| 21 | (comparative adj stud*).mp. | 764161 |
| 22 | etiology.mp. | 545024 |
| 23 | exp Cohort Studies/ or cohort.mp. | 504363 |
| 24 | or/10-23 [epidemiological surveys] | 6448395 |
| 25 | 3 and 8 and 9 and 24 | 3788 |

| | | |
|---|---|---|
| 26 | limit 25 to yr="1990 -Current" | 3434 |

| African Index Medicus database to 06 September 2015 | | |
|---|---|---|
| # | Search | Results |
| 1 | Fever, or (increased body temperature) or febrile or (high temperature) or hyperthermia or pyrexia or (elevated temperature) | 18 |
| 2 | Children or infant or newborn or small or postmature or premature or minor or children or pediatric or perinatured or neonat or newborn or infant or boy or girl or kid or paediatric | |
| 3 | 1990 or 1991 or 1992 or 1993 or 1994 or 1995 or 1996 or 1997 or 1998 or 1999 or 2000 or 2001 or 2002 or 2003 or 2004 or 2005 or 2006 or 2007 or 2008 or 2009 or 2010 or 2011 or 2012 or 2013 or 2014 or 2015 | |

# Chapter 3

## *Treatable causes of fever among children under 5 years in a seasonal malaria transmission area, Nanoro in Burkina Faso*

Francois Kiemde, Marc Christian Tahita, Palpouguini Lompo, Toussaint Rouamba, Athanase M. Some, Halidou Tinto, Petra F. Mens, Henk D. F. H. Schallig and Michael Boele van Hensbroek.

**Infectious diseases of Poverty 2018, 7: 60**

**Abstract**

**Background:** Fever remains a major public health problem. In Burkina Faso, more than half of febrile children are considered not to be infected by malaria. This study prospectively assessed probable (treatable) causes of fever in Burkinabe children.

**Methods:** A prospective study was conducted among febrile children (≥37.5 °C) under-5 years of age presenting at four health facilities and one referral hospital in rural Burkina Faso. From each participant, blood was collected for malaria microscopy and culture, urine for dipstick testing and culturing if tested positive for leucocytes and nitrite, stool for rotavirus/adenovirus testing, culture and parasitology, and a nasopharyngeal swab for culture.

**Results:** In total 684 febrile children were included in the study. *Plasmodium falciparum* malaria was found in 49.7% (340/684) of the participants and non-malaria infections in 49.1% (336/684) of children. The non-malaria infections included gastro-intestinal infections (37.0%), common bacterial pathogens of nasopharynx (24.3%), bacterial bloodstream infections (6.0%) and urinary tract infections (1.8%). Nearly 45% (154/340) of the malaria infected children were co-infected with non-malaria infections, but only 3.2% (11/340) of these co-infections could be considered as a possible alternative cause of fever. In contrast, in the malaria microscopy negative children 18.0% (62/344) of the infections could be the probable cause of the fever. Pathogens were not isolated from 23.7% (162/684) of the febrile cases.

**Conclusions:** Malaria remains the most common pathogen found in febrile children in Burkina Faso. However, a relative high number of febrile children had non-malaria infections. The correct diagnosis of these non-malaria fevers is a major concern, and there is an urgent need to develop more point-of-care diagnostic tests and capacities to identify and treat the causes of these fevers.

**Key words**: fever, children, infectious diseases, malaria.

**Background**

Febrile illnesses remain a major public health problem in sub-Saharan Africa (SSA). Fever is the most common clinical symptom found in children under-5 years of age presenting at health facilities (1) (2). Nowadays in many malaria endemic areas, including Burkina Faso, more than half of these febrile children are considered not to be infected with malaria, as the incidence of this disease is declining due to increased control efforts over the last decade (3) (4) (5) (6). This, combined with the guidelines of the World Health Organization (WHO) that recommend to confirm a malaria infection in febrile children through a diagnostic test before giving antimalarial treatment (7), prompts the need to search for an alternative cause of fever. This has created a diagnostic dilemma for health workers, by having a large group of patients with so called "unexplained" or "non-malaria" fever and with few or no diagnostic tools available to guide the subsequence management of these febrile cases (8) (9) (10). An alternative cause of fever may also be present in malaria positive children. Subsequently, lack of knowledge on the prevalence of other possible aetiologies of fever, limited access to proper diagnostic tests, as well as the fear of overlooking a potentially life threatening malaria infection are the main reasons why most febrile children still receive antimalarial treatment (11) (12) (13).

The various possible aetiologies of a febrile illness are difficult to distinguish when only information from medical history and physical examination are available (14) (15). Laboratory facilities are often scarce in health facilities in low-and middle-income countries (11) (16) (17). Therefore, fever is routinely treated in these countries using only clinical signs and symptoms (empiric treatment) (18) (19). Data are becoming available on the aetiologies of fever episodes in children under 5 years of age in SSA (20) (21) (22) (23) (24). It is noted that, next to malaria, viral infections are a very common cause of febrile disease (13) (21) (23), which cannot be treated with antibiotics. However, a significant number of febrile cases remain that do need treatment with appropriate antibiotics, but these treatable infections are often overlooked (20) (22). The clinician needs to know the possible presence of other treatable infections amongst malaria-infected and uninfected children presenting with fever.

The present prospective study was designed and conducted to identify the pathogens that could cause fever in children seeking care at five health centres in rural Burkina Faso. Febrile patients were evaluated for a wide range of infections, with a focus on treatable bacterial and parasitic infections, using conventional diagnostic. This study contributes to a better understanding of possible causes of fever in children under 5 years of age living in a malaria endemic region.

## Methods

### Study site

This study was conducted in the rural district of Nanoro, Burkina Faso. Four peripheral health facilities (Urbain, Godo, Nazoanga and Seguedin) and the referral hospital Centre Medical Saint Camille of Nanoro were involved in the data and clinical specimen collection. The choice of the peripheral health facilities was based on their accessibility for patients and their distance to the research laboratory (maximum of 20 km). The district hospital was included as recruitment centre in order to be able to capture also the more severely ill children who were referred from peripheral health facilities.

Malaria transmission in Nanoro district is hyper endemic and occurs principally between July and November. In 2010, the average under-5 mortality rate in Burkina Faso was 129/1000 life-births (25). Vaccination against *Haemophilus influenzae* type b was introduced into the extended program of immunization (EPI) in January 2006 (estimated coverage 86.8% in 2012 (26)) and against pneumococcal and rotavirus in October 2013 (Source: Ministry of Health, Burkina Faso).

### Study design

A prospective study was conducted from January-December 2015. The study was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014–11-130). All children under-5 years of age with an axillary temperature ≥37.5°C presenting at one of the participating health facilities or the referral hospital were asked to participate in the study. Written informed consent was obtained from parent or legal guardian prior to enrolment. The medical history and findings from clinical examination of the child were recorded on a standard Case Report Form (CRF) by trained study nurses. The following clinical specimens were collected from each participant: blood, urine, stool and a nasopharyngeal swab. Parents or adult guardians were asked to complete sample collection within 48 hours following inclusion when diagnostic specimen collection (stool and/or urine) could not be realized during the enrolment procedure. They were provided with instructions and sterile containers for that purpose. The parents or guardians were told to return the samples immediately after collection.

The participants were managed according to the national guidelines. If culture results or other laboratory tests justified additional or alternative treatment, the results were forwarded to the health facility. The parents or guardians were contacted as soon as possible to arrange that the necessary treatment would be given to the participant.

**Laboratory procedures**

Nasopharyngeal swab and blood samples were also collected in Skim Milk-Tryptone-Glucose-Glycerol (STGG) and Ethylene Diamine Tetra-Acetic Acid (EDTA) tube respectively. All samples were transported to the central microbiology laboratory of the Clinical Research Unit of Nanoro (CRUN) where the various laboratory tests were conducted.

**Malaria testing:** Giemsa stained thick and thin blood smears were prepared and independently read by two experienced microscopists. In case of discordance (i.e. positive *vs*. negative, difference in *Plasmodium* species, difference in parasite density >Log10 or ratio >2 in case of parasite density ≤400/µl or >400/µl) the respective slide was read by a third independent reader to make a final decision. The final parasite counts were the geometric means of the two reader's results or the geometric means of the two geometrically closest reading in case of third reading. These results were expressed as asexual parasites per microliter by using the patient's white blood cell (WBC) count.

**Full blood counts:** Blood samples were collected in EDTA tubes for full blood counts using a Sysmex XS1000i (Sysmex Corporation, Kobe, Japan) according to manufacturer's instructions.

**Blood culture:** From each child, 1–3 ml of venous blood was directly collected in a paediatric blood culture bottle (BD BACTEC Peds Plus™/F, Becton Dickinson and Company, Sparks, Maryland, USA) and incubated in a BACTEC 9050 instrument (Becton Dickinson) for a total of 5 days. If flagged for bacterial growth, the cultures were subsequently Gram stained, sub-cultured on Eosin-Methylene Blue (EMB) agar, 5% Sheep Blood agar (bioMérieux, Marcy-l'Etoile, France) and chocolate + isovitalex agar, and incubated at 35–37°C for 24 hours. Isolates were identified by standard microbiological methods.

**Stool examination:** Fresh stool smears were prepared in normal saline and examined by direct microscopy for the presence of intestinal parasites. Parasite elements looked for were: cysts, eggs, and vegetative forms of protozoa. Furthermore, stools were plated on EMB, Hektoen agar and inoculated in selenite of sodium broth and incubated at 35–37°C. After 4 hours of incubation, selenite of sodium broth was sub-cultured on *Salmonella* and *Shigella* agar (*SS* agar). To assess the prevalence of rotavirus and adenovirus after the introduction of vaccine against rotavirus in 2013, stool samples were also analysed for group A rotavirus using one step rotavirus and adenovirus serotype 40/41 in human faeces (SD Bioline Rota/Adeno; Standard Diagnostics Inc., Korea).

**Urine examination:** Dipstick testing (UroColor, Standard Diagnostics Inc, Korea) was done for each urine sample and the parameters collected were: presence or not of blood, protein, leucocytes and nitrite in the sample. A culture of the sample was done in case testing for leucocytes and nitrate was positive. Urine was plated on CLED (Cystine Lactose Electrolyte Deficient) and EMB agar when dipstick testing for leucocytes and nitrite was positive. The cultures were incubated at 35–37°C for 24 hours. Only samples that yielded pure bacterial growth of more than $10^5$ colonies forming units (CFU)/ml were considered significant bacteriuria.

**Nasopharyngeal specimen culture:** A flexible flocked swab was introduced into the nasal passage and two rotations of swabbing were done. The swab was placed into STGG broth for storage and transportation. For analysis, nasopharyngeal swab was vortexed and 200 µl of this broth was inoculated in 10 ml of Todd-Hewitt (TH) broth for enrichment and incubated at 35–37°C during 18 to 24 hours. The TH broth was subsequently plated out and incubated at 35–37°C for 24 hours on sheep blood agar and chocolate + isovitalex agar and chapman (mannitol-salt) agar to detect *Streptococcus* and/or *Staphylococcus* species, respectively.

**Interpretation of laboratory findings:** For the purpose of this study, the infections found, except malaria, were considered to be non-malaria infections (NMIs). The NMIs were further classified as either "probable causes of fever" or "non-probable causes of fever". The infections that could be considered as a "probable cause of fever" in the recruited cases were subsequently further regrouped in: bacterial bloodstream infections (bBSI), viral gastro-intestinal (vGII) caused by rotavirus or adenovirus, and urinary tract infections (UTI) (27) (28) (29) (30). The infections that could be considered as "non-probable cause of fever" in the study cases, were regrouped in: bacterial gastro-intestinal infection (bGII), common bacterial pathogens of the nasopharynx (CBPN) and parasitic gastro-intestinal infection (pGII), except for amoeba.

**Data analysis**

Data management involved double data entry using OpenClinica software. Data analysis was done by using R software version 3.3.1 (R development core team 2016, R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were summarized as proportions and Pearson's Chi-square test or Fisher's exact test were performed. Continuous variables were described by mean or median and compared by using the Student's t-test. Binomial logistic regression was used to compare the relation between the presence of infection and demographic, clinical and laboratory data. The P value $< 0.05$ was considered as significant.

## Results

### Characteristic of the study population

A total of 1447 children under-5 years of age, attending one of the participating health facilities or the referral hospital during the year 2015, were screened. 796 (55.0%) children were febrile (axillary temperature ≥37.5°C) and 684 febrile children (85.9%) were included in the study. The study flow chart is presented in figure 1. Reasons for exclusion were: residence outside the health facilities catchment areas or failing to obtain consent from parents or guardians. The mean age of the recruited children was 22.4 months (Standard deviation (SD): 14.1) and 29.4% were under 12 months of age. Mean axillary body temperature was 38.7°C (37.5–41.0°C) (Table 1).

Clinical specimens for blood culture and malaria microscopy were obtained from all study cases. The proportion of stool and urine samples collected was 76.5% (523/ 684) and 74.1% (507/684), respectively. A small proportion (61/684, 8.9%) of nasopharynx cultures could not be performed, because the training of study nurses to collect nasopharyngeal swabs was completed after the start of inclusion (Fig. 1 and Table 2).

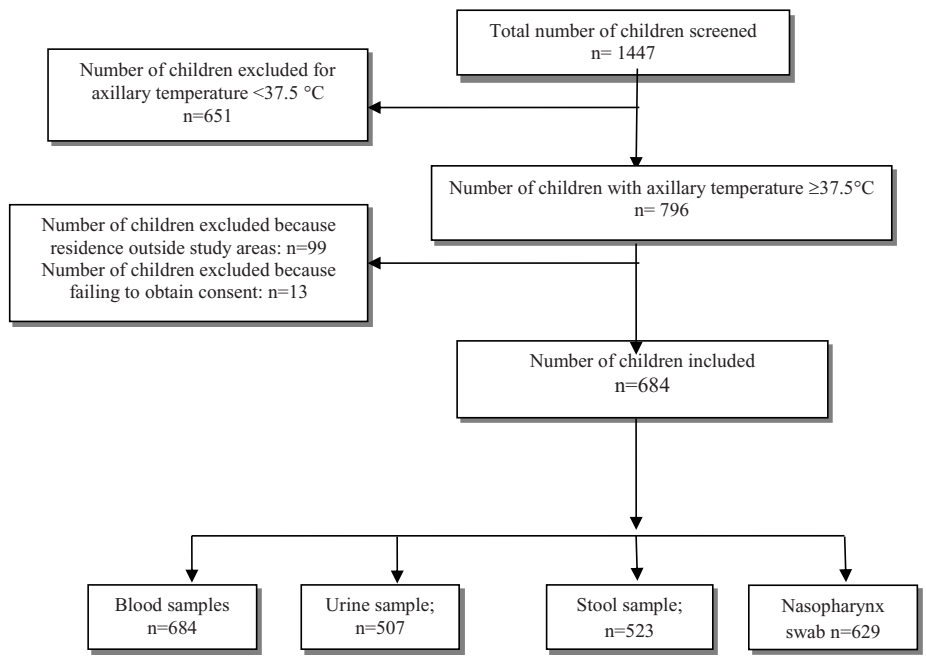


**Figure 1:** Study flow chart

**Table 1: Demographic characteristics, clinical symptoms and basic laboratory findings of study subjects enrolled in the present study in four rural health centres and one referral hospital in Nanoro Health district.**

| Category | Subcategory | *N* | Proportion (%) |
|---|---|---|---|
| **Gender** | Male | 369 | 53.95 |
| | Female | 315 | 46.05 |
| **Age** | ≤12 months | 201 | 29.39 |
| | >12 months | 483 | 70.61 |
| **Temperature** | ≥37.5 °C – ≤38.5 °C | 340 | 49.71 |
| | >38.5 °C – ≤39.5 °C | 242 | 35.38 |
| | >39.5 °C | 102 | 14.91 |
| **Duration of fever** | 1 day | 108 | 15.79 |
| | 2 – 3 days | 518 | 75.73 |
| | 4 – 7 days | 56 | 8.19 |
| | >7 days | 2 | 0.29 |
| **Vaccination according to the EPI** | Yes | 449 | 65.64 |
| | No | 133 | 19.44 |
| | Unknown | 102 | 14.91 |
| **Common symptoms** | Cough | 335 | 48.98 |
| | Diarrhoea | 266 | 38.89 |
| | Vomiting | 123 | 17.98 |
| | Others | 55 | 8.04 |
| **Basic laboratory findings** | **Haemoglobin rate** | | |
| | < 8 g/dl | 180 | 26.32 |
| | ≥ 8 g/dl – <11 g/dl | 418 | 61.11 |
| | ≥11 g/dl | 86 | 12.57 |
| | **White blood cells** | | |
| | <4×10$^3$ cells/mm$^3$ | 10 | 1.46 |
| | ≥4×10$^3$ cells/mm$^3$ – < 12.10$^3$ cells/mm$^3$ | 348 | 50.88 |
| | ≥12×10$^3$ cells/mm$^3$ | 326 | 47.66 |

EPI: Expanded program of immunization

### Prevalence of malaria and other pathogens in the febrile study cases

Malaria (confirmed by positive microscopy) was found in 49.7% (340/684) of the cases. Malaria occurred whole year round (Fig. 2). All 340 malaria microscopy positive cases were infected with *Plasmodium falciparum,* but five children were co-infected with other *Plasmodium*-species (i.e. 4 with *P. malariae* and 1 with *P. ovale*). The geometric mean parasite density was 24,003 parasites/µl (range: 72–2,272,500). Malaria was more prevalent in children presenting at the health facilities than at the referral hospital (Table 2).

Bacteria could be identified in 6.0% (41/684) of blood cultures, 2.0% (10/507) urine cultures, 7.7% (40/523) stools and 24.3% (154/623) nasopharyngeal cultures. Parasites were found in 27.3% (143/523) of the collected stool specimen and 4.8% (25/523) of these samples were positive for rotavirus/adenovirus. Pathogens could not be detected in 162 children (23.7% of the study population) (Table 2).

A pathogen (other than malaria) was identified in 10.7% (73/684) of the febrile children that could be "a probable cause of fever" (Table 2). Of these pathogens, 6.0% (41/684) was a

bacterial bloodstream infection (bBSI), 4.8% (25/253) was a viral gastro-intestinal tract infection (vGII) and 2% (10/507) was a urinary tract infection (UTI). Only mono-infections were found in the bacterial cultures (bBSI and UTI). Non-typhoid *Salmonella* was the dominant bacterium isolated from positive blood cultures (75.6%, 31/41) and all ten positive urine cultures contained *E. coli* (10/10). Rotavirus was the main virus detected in positive stools tested with rotavirus/adenovirus tests (60%, 15/25). Pathogens that could be "a probable cause of fever" (bBSI and UTI, except for vGII) were more often found in clinical specimen from children presenting at the referral hospital than in those presenting at the peripheral health facilities (Table 2).

In 40.8% (279/684, i.e. 143 children in the malaria microscopy positive group and 136 children in the malaria microscopy negative group) of the febrile children one or more pathogens were identified that could be considered as "non-probable cause of fever" (Table 3). Parasites were detected in 27.3% (143/523) of the stool samples. *Giardia intestinalis* was the most frequently found parasite in stool samples 46.2% (66/143). Pathogenic bacteria were isolated from 7.7% (40/523) of the stool samples (Table 2). The most often isolated bacterial species from stool was *non-typhoid Salmonella* 50.0% (20/40). Nasopharyngeal swab cultures were positive for 24.3% (153/629) of the febrile cases. *Staphylococcus aureus* was the most frequently found species 96.7% (148/153) amongst the common bacterial pathogens of nasopharynx (CBPN). The prevalence of pathogens that were probably not the cause of fever was more or less similar in clinical specimen from children presenting at the referral hospital and in those presenting at the peripheral health facilities (see Table 2).

All pathogens were found the whole year round except for UTI. Malaria followed more or less the typical seasonal distribution for the study area, with peak transmission between August and January (Figure 2). CBPN were not studied during the first two months of the study. UTIs were found mainly during the second half of the year (Figure 2).

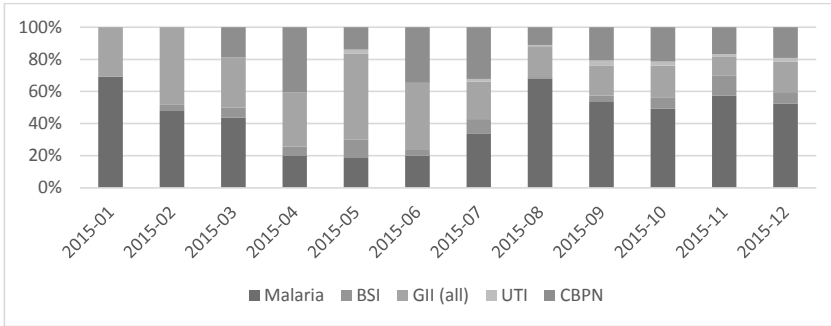


**Figure 2: Distribution of pathogens found with microbiology during the whole study (year 2015)**
BSI: Bloodstream infection (bacterial); GII: Gastro-intestinal infection (all); UTI: Urinary tract infection; CBPN: common bacterial pathogens of nasopharynx

**Table 2: Microbiology and malaria microscopy findings of children under-5 years enrolled in this study**

| Subcategory | General population *n*/*N* (%) | Referral hospital *n*/*N* (%) | Health facilities *n*/*N* (%) |
|---|---|---|---|
| **Malaria microscopy** | 684/684 (100) | 138/138 (100) | 546/546 (100) |
| **Malaria microscopy positive** | 340/684 (49.71) | 41/138 (29.71) | 299/546 (54.76) |
| ***Plasmodium falciparum*†** | 340/340 (100) | 41/138 (29.71) | 299/546 (54.76) |
| ***Plasmodium malariae*** | 04/340 (0.88) | 0/138 (0) | 4/299 (1.34) |
| ***Plasmodium ovalae*** | 01/340 (0.29) | 0/138 (0) | 1/299 (0.33) |
| **Blood cultures (*N* = 684)** | 684/684 (100) | 138/138 (100) | 546/546 (100) |
| **Bacterial culture positive** | 41/684 (5.99) | 21/138 (15.22) | 20/546 (3.66) |
| ***Non-Typhoid Salmonella* ssp** | 31/41 (75.61) | 19/21 (90.48) | 12/20 (60.00) |
| ***Salmonella typhi*** | 02/41 (4.88) | 00 | 02/20 (10.00) |
| ***Escherichia coli*** | 01/41 (2.44) | 00 | 01/20 (5.00) |
| ***Staphylococcus aureus*** | 02/41 (4.88) | 01/21 (4.76) | 01/20 (5.00) |
| ***Streptococcus pneumoniae*** | 03/41 (7.31) | 01/21 (4.76) | 02/20 (10.00) |
| ***Neisseria meningitidis*** | 02/41 (4.88) | 00 | 02/20 (10.00) |
| | | | |
| **Urine examination (*N* = 507)** | 507/684 (74.12) | 94/138 (68.12) | 413/546 (75.64) |
| **Bacterial culture positive** | 10/507 (1.97) | 04/94 (4.26) | 06/413 (1.45) |
| ***Escherichia coli*** | 10/10 (100.00) | 04/4 (100) | 06/6 (100) |
| | | | |
| **Stool examinations (*N* = 523)** | 523/684 (76.46) | 85/138 (61.60) | 438/546 (80.22) |
| **Bacterial culture positive** | 40/523 (7.65) | 09/85 (10.59) | 31/438 (7.08) |
| ***Non-Typhoid Salmonella*** | 20/40 (50.00) | 08/9 (88.89) | 16/31 (38.71) |
| ***Escherichia coli*** | 17/40 (42.50) | 01/9 (11.11) | 16/31 ()51.61 |
| ***Shigella*** | 03/40 (7.50) | 00 | 03/31 (9.69) |
| **Parasites positive** | 143/523 (27.34) | 17/85 (20.00) | 126/438 (28.77) |
| **Mono parasitosis††** | 115/143 (80.42) | 11/17 (64.71) | 104/126 (82.54) |
| **Bi parasitosis** | 25/143 (17.48) | 05/17 (29.41) | 20/126 (15.87) |
| **Tri parasitosis** | 03/143 (2.10) | 01/17 (5.88) | 02/126 (1.59) |
| **Rotavirus/Adenovirus positive** | 25/523 (4.78) | 04/85 (4.71) | 21/438 (4.80) |
| **Rotavirus** | 15/25 (60.00) | 00 | 15/21 (71.43) |
| **Adenovirus** | 10/25 (40.00) | 04/4 (100) | 06/21 (28.57) |
| | | | |
| **Nasopharyngeal cultures (*N* = 629)** | 629/684 (91.95) | 137/138 (99.28) | 492/546 (90.11) |
| **Bacterial culture positive** | 153/629 (24.32) | 41/137 (29.71) | 112/492 (22.77) |
| ***Staphylococcus aureus*** | 148/153 (96.73) | 41/41 (100) | 107/112 (95.54) |
| ***Streptococcus pneumoniae*** | 05/153 (3.27) | 00 | 05/112 (4.46) |
| | | | |
| **No pathogens detected** | 162/684 (23.68) | 40/138 (28.99) | 122/546 (22.34) |

†: 5 children infection with *Plasmodium falciparum* were co-infected with *P. malariae* and *P. ovale*.
††: *Giardia intestinalis*: 35.65%; *Trichomonas intestinalis*: 22.61%; *Endolimax nana*: 17.40%; *Entamoeba coli*: 13.91%; *Entamoeba hystolitica*: 7.82%; Others: 2.61%.

**Table 3: Interaction between malaria and others pathogens studied**

| | Malaria microscopy positive (340) *n* (%) | Malaria microscopy negative (344) *n* (%) |
|---|---|---|
| **Other infections** | 154 (45.29) | 182 (52.91) |
| **Probable cause of fever (BSI, vGII, UTI)** | 11 (3.23) | 62 (18.02) |
| **No probable causes of fever (pGII, bGII, CBPN)** | 143 (42.05) | 136 (39.53) |
| **bBSI** | 6 (1.76) | 35 (10.17) |
| **GII (all)** | 90 (26.47) | 103 (29.94) |
| **pGII** | 77 (22.64) | 66 (19.18) |
| **bGII** | 17 (5.0) | 23 (6.69) |
| **vGII** | 3 (0.88) | 22 (6.40) |
| **CBPN** | 75 (22.05) | 78 (22.67) |
| **UTI** | 3 (0.88) | 7 (2.03) |

bBSI: bacterial bloodstream infection; GII: gastro-intestinal infection; pGII: parasitic gastro-intestinal infection; bGII: bacterial gastro-intestinal infection; vGII: viral gastro-intestinal infection; UTI: Urinary tract infection; CBPN: common bacterial pathogens of nasopharynx

### Interaction between malaria and other pathogens

Additional pathogens, next to *Plasmodium,* were isolated from 45.3% (154/340) of the clinical samples obtained from children who were malaria microscopy positive (Table 3). Only 11/154 (7.1%) of these other pathogens were identified as a pathogen that could also be considered as a "probable cause of fever". Consequently, 3.2% (11/340) of the malaria microscopy positive children were co-infected with other pathogens that could be a "probable cause of fever". In the malaria microscopy negative group 52.9% (182/344) of other infections were found. A significant higher number of these pathogens, 18.0% (62/344), was identified as a "probable cause of fever" in the malaria negative group of children compared to the malaria infected cases ($p < 0.001$).

### Correlation between the presence of infection and demographic, clinical and laboratory data

The correlation between the presence of an infection investigated in this study and the patient's demographic, clinical and laboratory data is summarized in table 4. The analysis showed a strong correlation between having a malaria infection and age >12 months (OR = 2.85, 95% CI = 2.0–4.1, $p<0.001$) or temperature >39.5 °C (OR = 2.06, 95% CI = 1.3–3.3, $p=0.002$). Furthermore, malaria was associated with white blood cell count $>12.10^3/mm^3$, and haemoglobin concentration <11g/dl (see table 4).

Only age >12 months was significantly associated with GII (OR = 2.9, 95% CI = 1.9–4.6, $p<0.001$). A correlation between UTI or CBPN and the risk factors studied was not found.

**Table 4: Correlation between malaria, BSI, UTI, GII and CBPN and demographic, clinical and laboratory data.**

| | Malaria | | Blood stream infection | | Urinary tract infection | | Gastro-intestinal infection | | CBPN | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *OR* (95% *CI*) | *P*-value | *OR* (95% *CI*) | *P*-value | *OR* (95% *CI*) | *P*-value | *OR* (95% *CI*) | *P*-value | *OR* (95% *CI*) | *P*-value |
| **Gender** | | | | | | | | | | |
| **Male** | 1 | | 1 | | 1 | | 1 | | 1 | |
| **Female** | 1.19 (0.88–1.61) | 0.255 | 1.01 (0.53–1.91) | 0.969 | 1.22 (0.34–4.45) | 0.752 | 1.18 (0.83–1.69) | 0.350 | 1.18 (0.82–1.70) | 0.381 |
| **Age** | | | | | | | | | | |
| **≤12 months** | 1 | | 1 | | 1 | | 1 | | 1 | |
| **>12 months** | 2.85 (2.02–4.06) | <0.001 * | 0.70 (0.37–1.39) | 0.299 | 0.34 (0.09–1.25) | 0.094 | 2.88 (1.86–4.58) | <0.001 * | 0.90 (0.61–1.35) | 0.609 |
| **Temperature** | | | | | | | | | | |
| **[37.5 °C – 38.5 °C]** | 1 | | 1 | | 1 | | 1 | | 1 | |
| **[38.5 °C – 39.5 °C]** | 1.26 (0.91–1.76) | 0.166 | 1.28 (0.64–2.51) | 0.479 | 1.93 (0.50–7.92) | 0.330 | 0.99 (0.67–1.47) | 0.963 | 1.21 (0.81–1.80) | 0.334 |
| **>39.5 °C** | 2.06 (1.31–3.26) | 0.002* | 0.87 (0.28–2.23) | 0.789 | 0.86 (0.04–5.90) | 0.891 | 1.13 (0.67–1.89) | 0.647 | 0.90 (0.50–1.55) | 0.714 |
| **White cell counts** | | | | | | | | | | |
| **[4×10³–12×10³]** | 1 | | 1 | | 1 | | 1 | | 1 | |
| **<4×10³ cells/mm³** | 1.18 (0.33–4.67) | 0.803 | 0.35 (0.02–7.31) | 0.828 | 0.66 (0.20–26.68) | 0.828 | 1.55 (0.36–6.70) | 0.540 | 1.13 (0.01–2.31) | 0.166 |
| **≥12×10³ mm³** | 0.58 (0.43–0.79) | <0.001 * | 1.03 (0.55–1.91) | 0.206 | 2.25 (0.64–7.92) | 0.206 | 0.79 (0.55–1.14) | 0.211 | 1.36 (0.94–1.95) | 0.100 |
| **Anaemia** | | | | | | | | | | |
| **≥11 g/dl** | 1 | | 1 | | 1 | 1 | 1 | | 1 | |
| **<8 g/dl** | 4.06 (2.35–7.20) | <0.001 * | 1.60 (0.54–5.80) | 0.426 | 1.50 (0.31–10.70) | 0.631 | 1.71 (0.39–1.28) | 0.252 | 1.16 (0.64–1.18) | 0.625 |
| **[8 g/dl–11 g/dl]** | 2.51 (1.53–4.24) | <0.001 * | 1.25 (0.47–4.33) | 0.688 | 0.34 (0.06–2.65) | 0.248 | 0.60 (0.35–1.01) | 0.052 | 0.81 (0.46–1.44) | 0.453 |
| **Vaccination according EPI** | | | | | | | | | | |
| **Yes** | 1 | | 1 | | 1 | | 1 | | 1 | |
| **No** | 0.78 (0.52–1.14) | 0.204 | 0.68 (0.25–1.58) | 0.409 | 0.12 (0.01–2.05) | 0.145 | 1.23 (0.81–1.98) | 0.294 | 0.98 (0.60–1.58) | 0.950 |
| **Unknown** | 1.32 (0.86–2.05) | 0.204 | 0.90 (0.33–2.10) | 0.829 | 0.16 (0.01–2.66) | 0.201 | 1.11 (0.64–1.88) | 0.709 | 1.06 (0.63–1.73) | 0.829 |

**P*-value is statistically significant

BSI: Blood stream infection; UTI: Urinary tract infection; GII: Gastro-intestinal infection; CBPN: Common bacterial pathogens of nasopharynx

**Discussion**

This study demonstrated that *P. falciparum* causing malaria is still the main pathogen identified in febrile children in the Nanoro region in Burkina Faso. As malaria can be relatively easily diagnosed, under-or overtreatment of this disease could be avoided if health workers adhere to the results of diagnostic testing (6) (31). Next to malaria, a very small proportion of the febrile malaria-positive children were also co-infected with another potentially fever causing pathogen, such as a bacterial bloodstream infection (bBSI), which can cause a prolonged fever after successful malaria treatment. More importantly, if malaria is ruled out as the attributable cause of fever, the alternative cause fever should be established taking into account that in about 20% of the febrile malaria-negative children the actual cause of fever could either be a bBSI or a viral gastro-intestinal infection (vGII) or a urinary tract infection (UTI).

The prevalence and distribution of possible causes of fever found in the present study are in line with other studies performed in SSA in the same age group (29) (32) (33) (34) (35) (36) (37). Importantly, the study re-emphasizes that a proper diagnosis of the cause of the fever episode in children in this age group followed by appropriate treatment is needed, as it will obviously save lives and prevent incorrect prescription of drugs. Only malaria can easily be diagnosed as a possible cause of fever in most health facilities in low and middle income countries at present. Therefore, there is a pressing need to develop practical tools (point-of-care or near point-of-care) to diagnose other treatable causes of fever in order to reduce morbidity and mortality in this age group (6).

Non-malaria infections that probably contributed to fever in this study were bBSI, UTI and vGII. The prevalence of bBSI was, despite differences in study designs, comparable to those reported in other studies in SSA (4 to 10%) (32) (38) (39) (40). The severely ill cases were referred to the referral hospital Centre Medical Saint Camille. This explains why bBSI and UTI cases were more prevalent in the referral hospital than in the rural health facilities. The high prevalence of Gram negative bacteria in general, and non-typhoid *Salmonella* in particular, is in agreement with a previous study in the same area, and with the global trend. (41). UTI found in this study was less prevalent than reported in studies from Tanzania and Nigeria. However, the predominant bacteria causing UTI in Nigeria and Tanzania were *Escherichia coli* (as in this study) (22) (28) (42) (43) (44). In general, the hygienic conditions and sanitation are relatively good in the present study area and children are often well supervised by their parents or guardian, which could explain the low prevalence of UTI observed in this study. Around

5% of the febrile children were infected by vGII caused by rotavirus and adenovirus. This proportion is lower than that previously reported (33.8%) in Ouagadougou before the introduction of the rotavirus vaccine in Burkina Faso in 2013 (36). The low prevalence observed in the current study could therefore be attributed to the rotavirus vaccination program in place in Burkina Faso. Contamination of stool cultures was not reported. However, 6.3% (32/507) of the urine samples collected by parents or guardians were contaminated. Urine collection is a rather difficult process, in particular to avoid contamination, and parents/guardians were not trained to do this and this explains the relative high rate of urine contaminations. Only 0.4% (3/684) of the blood samples collected by the nurses were contaminated.

This study also identified the presence of pathogens that can be considered as "non-probable cause of fever", such as gastro-intestinal parasites, common bacterial pathogens of the nasopharynx and bacteria in stool. Still these pathogens require attention in patient management as they may affect health, for example in case the protective mucosal barrier of the intestine or nasopharynx is broken and they enter the blood stream (30) (45). A limitation of the study is that viral respiratory tract infections, which are known to be a cause of fever (13) (21) (22) (23) (46) (47) (48) were not studied. These viral infections of the respiratory tract do not necessarily need treatment, and their detection require sophisticated laboratory facilities. As other studies have demonstrated that respiratory tract viral infections can be a major attributor to fever (21) (22) (23), it might be possible that these pathogens are the cause of febrile illness in the children in which no probable causes of fever could be identified. The study revealed some correlations between the presence of an infection investigated and the patient's demographic, clinical and laboratory data. In particular a relation between having malaria and moderate to severe anaemia was found, which is in line with previous findings in Nigeria and Ghana (49) (50). Furthermore, children with a normal WBC had a higher risk of being infected with malaria than those having a high WBC (51). A high temperature or high WBC counts was not predictive for a bacterial infection in the present study, which is in contrast to previous findings (39). Significant other correlations were not found.

**Conclusion**

This study showed that malaria remains strongly associated with paediatric fever episodes in Nanoro. A relatively high number of non-malaria infections (NMIs) was found in malaria microscopy positive as well as malaria microscopy negative febrile children. Among these NMIs, a "probable cause of fever" was mainly found in the malaria-negative febrile children.

As treatment of several of these NMIs is possible, there is a need for a practical tool to help clinician to screen for treatable causes of the febrile episodes. In the absence of such a diagnostic tool, malaria diagnostic test can be of help for the clinician to decide who should receive additional treatment, but over- or under- treatment will remain likely and puts febrile children at significant risk of morbidity and mortality.

**List of abbreviations**

bBSI: bacterial bloodstream infection

bGII: bacterial gastro-intestinal infection

CBPN: common bacteria pathogen of nasopharynx

CFU: colonies forming units

CLED: cysteine lactose electrolyte deficient

CRF: case report form

CRUN: clinical research unit of Nanoro

EDTA: ethylene diamine tetra acetic acid

EMB: eosin methylene blue

EPI: extended program of immunization

Log: logarithm

NMIs: non-malaria infections

pGII: parasitic gastro-intestinal infection

SD: standard deviation

*SS*: *Salmonella* and *Shigella*

SSA: sub-Saharan Africa

STGG: skim milk-tryptone-glucose-glycerol

TH: todd-hewitt

UTI: urinary tract infection

vGII: viral gastro-intestinal infection

WBC: white blood cells

WHO: world health organization

**Declarations**

**Ethics approval and consent to participate**

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent was obtained from parents or guardians for the participation of the children prior to enrolment in the study.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

FK, HS, PM, HT and MBvH conceived and designed the study. FK, AS and MT supervised patient inclusion, taking of informed consent and diagnostic specimen collection by study nurses. KF, PL and MT performed the laboratory analyses. FK and TR analysed the data. FK, HS and MBvH drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript

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# *Chapter 4*

## *Implementation of a malaria rapid diagnostic test in a rural setting of Nanoro, Burkina Faso: from expectation to reality*

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**Abstract**

**Background:** Malaria rapid diagnostic tests (RDTs) are nowadays widely used in malaria endemic countries as an alternative to microscopy for the diagnosis of malaria. However, quality control of test performance and execution in the field are important in order to ensure proper use and adequate diagnosis of malaria. The current study compared the performance of a histidine-rich protein 2-(HRP2) based RDT used at peripheral health facilities level in real life conditions with that performed at central reference laboratory level with strict adherence to manufacturer instructions.

**Methods:** Febrile children attending rural health clinics were tested for malaria with a RDT provided by the Ministry of Health of Burkina Faso as recommended by the National Malaria Control Programme. In addition, a blood sample was collected in an Ethylene Diamine Tetra Acetic acid (EDTA) tube from all study cases for retesting with the same brand of RDT following the manufacturer's instructions with expert malaria microscopy as gold standard at the central reference laboratory. Fisher exact test was used to compare the proportions by estimating the p-value ($p \leq 0.05$) as statistically significant

**Results:** In total, 407 febrile children were included in the study and malaria was diagnosed in 59.9% (244/407) of the cases with expert malaria microscopy. The sensitivity of malaria RDT testing performed at health facilities was 97.5% and comparable to that achieved at the laboratory (98.8%). The number of malaria false negatives was not statistically significant between the two groups ($p=0.5209$). However, the malaria RDT testing performed at health facilities had a specificity issue (52.8%) and was much lower compared to RDT testing performed at laboratory (74.2%). The number of malaria false positives was statistically significantly different between the two groups ($p=0.0005$).

**Conclusion:** Malaria RDT testing performed at the participating rural health facilities resulted in more malaria false positives compared to those performed at central laboratory. Several factors, including storage and transportation conditions but also training of health workers, are most likely to influence test performance. Therefore, it is very important to have appropriate quality control and training programmes in place to ensure correct performance of RDT testing.

**Key words:** malaria, HRP-2, RDT, microscopy, sensitivity and specificity.

**Background**

The National Malaria Control Programme (NMCP) guidelines in Burkina Faso recommend that all suspicious malaria cases should be confirmed using either a RDT or light microscopy (if available) (1) (2). Microscopy detecting *Plasmodium* parasites in Giemsa stained thick or thin blood slides still remains the gold standard for malaria diagnosis (3). The sensitivity and specificity of microscopy is however depending on the quality of the blood films, maintenance of the microscopy and training of the microscopists. Therefore, in many peripheral health settings RDTs have been introduced to fill this gap (4). Malaria RDTs are in principle easy to perform in the field outside of a conventional laboratory, do not need much training to be performed, are relative cheap, and give a diagnostic result within 15 minutes (5).

The decision of many countries of sub-Saharan Africa (SSA), including Burkina Faso, to select malaria rapid diagnostic test detecting *Plasmodium falciparum*-specific histidine-rich protein 2 (*Pf*HRP2) for diagnosis malaria is based on the high sensitivity and specificity reported by the World Health Organization (WHO) and Foundation for Innovative New Diagnostics (FIND) malaria RDT evaluation programme (6). Secondly, HRP2-based RDTs are reported to have a good thermal and humid stability compared to tests targeting *Plasmodium*-specific parasite lactate dehydrogenase (pLDH) (7). Indeed, exposure of *Pf*HRP2 RDT to high temperature (up to 45°C) over a prolonged period of up to 24 months did not affect the quality of the test (6). However, the influence of a very high temperature (> 45°C; which is common in several SSA) and humidity (>65%) are not documented. In addition, transport and storage conditions and operator performance have been reported to influence RDT performance (8) (9) (10) (11). Thirdly, the issue of persisting HRP2 antigens after successful treatment has been raised as a major factor contributing to reduce the specificity of *Pf*HRP2-based RDTs (12) (13) (14) (15) (16) (17) (18) (19). Finally, there is also increasing concern with respect to reported false positive diagnosis by *Pf*HRP2 RDT in particular in low malaria transmission settings (20).

These concerns warrant close monitoring of the performance of RDTs under field conditions. The objective of the present study was to assess the performance of the recommended HRP2 RDT by the Burkina Faso NMCP executed at peripheral level compared with the performance of the same brand of RDT in controlled conditions at a central reference laboratory with strict adherence to the manufacturer instructions.

## Methods

### Study design

The study was conducted between April and October 2016 in the health district of Nanoro, which is located at approximately 100 km from Ouagadougou, the capital city of Burkina Faso. Malaria is endemic with the transmission peak occurring between July and November and *Plasmodium falciparum* is the predominant malaria parasite (21). The study was conducted as part of a large project aiming to assess fever aetiologies in Nanoro (22). Briefly, children under 5 years with an axillary temperature ≥37.5 °C presenting at one of the participating health facilities were asked to participate. After obtaining the consent from parent or legal guardian, the participant was enrolled in the study. The malaria RDT used to screen febrile children at recruitment in the health facilities during the study period was the HRP2-based RDT specific to *P. falciparum* (SD Ag Bioline *Pf*: Standard Diagnostics, Hagal-Dong, Korea). Information on lot number and expiration date was not collected. The result of malaria RDT testing in the health facilities was recorded on a case record form.

After inclusion, a blood sample was collected for each child in ethylene diamine tetra acetic acid (EDTA) tube, transported under cold conditions in an ice-box at the laboratory of Clinical Research Unit of Nanoro (CRUN). Expert malaria microscopy was performed by expert laboratory technician from blood collected in EDTA tube before stored at -20°C until retesting. The retesting is done at the laboratory of CRUN with experimented technician with a *Pf*HRP2 RDT of the same manufacturer (SD Ag Bioline *Pf*: Standard Diagnostics, Hagal-Dong, Korea: Lot number: 05EDC002A; Expiration date: 01/03/2019). The RDTs are transported and stored according to the manufacturer's instructions. Standard Operating Procedures (SOPs) for ordering, transportation, storage and performing malaria RDTs are in place at CRUN. Blood samples are thawed at room temperature before retesting.

### Laboratory procedures

For retesting in the CRUN laboratory, the blood sample was thawed at room temperature and the diagnostic test was performed according to the manufacturer's instructions. One trained technician performed the RDT, but the result was read by two technicians and in case of a discordant opinion a third reader would be consulted. The laboratory technicians who repeated the malaria RDT were blinded from the malaria RDT results obtained at the health facilities and the RDT test results were reported on separate case record forms.

Malaria slide reading was performed by expert microscopists who are participating in an external quality programme and only certified microscopists were allowed to read the slides. The limit of detection (LoD) of this expert microscopy was 10 parasites per µl (23). Thin films were fixed with methanol and blood slides were stained with 3% Giemsa solution (pH 7.2) for identification and quantification of asexual *P. falciparum* and other *Plasmodium* species. Parasites densities were determined by counting the number of asexual parasites per 200 white blood cells, and calculating per µl of blood by assuming the number of white blood cells to be at 8000 per µl. Thick blood smears were considered negative when the examination of 200 fields per thick film did not reveal the presence of any asexual parasites. Each blood slide was read by two independent expert readers, and in case of discordance (positive *vs* negative, different in *Plasmodium* species, difference in parasite density >Log10 or ratio>2 in case of parasite density ≤400/µl or >400/µl, respectively), the blood slide was read by a third independent reader. Positive microscopy results were recorded as the geometric means of the two reader's results or the geometric means of the two geometrically closest reading in case of third reading. These results were expressed as asexual parasites per µl by using the patient's white blood cell (WBC) count. A selection of slides (5%) was re-read by an independent expert microscopist for quality assurance. All microscopists were blinded from the results obtained with the different malaria RDTs.

Ethical approval

The study was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). The study was also approved by the health district authorities and community leaders of different villages before implementation.

**Data analysis**

Double entered data was done using Excel 2016. The data analysis was done with R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For the quantitative data, the descriptions were performed by using mean or median respectively. Proportion was used to describe qualitative data. The evaluation of the performance of the malaria RDT performed by nurses at health centres level was done by comparing the results of *Pf*HRP2 tests performed in the field by health facilities nurses to those repeated in CRUN laboratory by trained technicians. Proportions were used to present the concordance and discordance between the two tests performed in malaria positive and negative groups. Fisher exact test was used to compare the proportions by estimating the p-value ($p \leq 0.05$) as statistically significant.

Agreement between each RDT test and microscopy and between the two RDTs tests was determined by calculating Kappa (κ) values with 95% confidence intervals by using GraphPad software (https://www.graphpad.com/quickcalcs/).

**Results**

*Description of the study population*

In total, 407 children were included in the study. The median age of enrolled children was 23.0 months [IRQ (interquartile): 12.0–36.0] and the mean axillary temperature was 37.7 °C (standard deviation: 0.78°C). Males represented 56.8% (231/407) of the study population (Table 1).

**Table 1: Baseline characteristic of study population**

| Characteristics | N= 407 |
|---|---|
| Age in months, median (IQR) | 23.0 (12.0-36.0) |
| **Male, n (%)** | 231 (56.8) |
| **Axillary** Temperature °C, mean (SD) | 37.7 (0.78) |
| Parasites/µl, geometric mean (min-max) | 22,839. 4 (32-586,250) |
| **Malaria positive by expert microscopy, n (%)** | 244 (59.9) |
| Malaria positive health facilities RDT-*Pf*HRP2, n (%) | 315 (77.4) |
| Malaria positive laboratory RDT-*PfHRP2*, n (%) | 282 (69.3) |

*Results of RDT testing and expert microscopy*

The number of positive cases of malaria determined by performing a *Pf*HRP2 RDT at the health facilities level by nurses (HF-*Pf*HRP2) was 77.4% (315/407). No test failures were reported at health facilities. Retesting the collected blood samples in the laboratory of CRUN (Lab-*Pf*HRP2) revealed that 69.3% (282/407) was RDT positive (Table 1). No invalid tests were observed at CRUN and there was no need for a third opinion as all initial readings were in agreement.

The number of *P. falciparum* malaria microscopy positive slides as assessed by expert microscopists was 59.9% (224/407), with a geometric mean and median parasite density of 22,839.4 parasites/µl (range: 32–586,250 parasites/µl) and 39,847 (7828–95,369), respectively. Co-infections were found in 5 cases: there were 4 co-infections with *Plasmodium malariae* and 1 case with *Plasmodium ovale*. There was no need for a third reader as the two expert technicians agreed on the initial microscopy result.

*Performance of malaria RDT detecting PfHRP2 performed at the health facilities level by nurses and at the laboratory level by trained technicians compared to expert microscopy*

The number of malaria true positive cases (by considering expert microscopy as the gold standard) was 58.5% (238/407) with the *Pf*HRP2 RDT supplied by the NMCP and performed by the nurses at health facilities level (HF-*Pf*HRP2) and 59.0% (240/407) with the *Pf*HRP2 RDT purchased by CRUN and performed by trained laboratory technicians at laboratory level (Lab-*Pf*HRP2) (Table 2). This difference was not statistically significant between the two groups (p=0.8848), as well as the number of false negatives was not statistically different between the two groups [1.5% (6/407) *versus* 1.0% (4/407); p=0.5209]. However, the number of malaria true negatives was significantly different between HF-*Pf*HRP2 and Lab-*Pf*HRP2 [21.1% (86/407) *versus* 29.7% (121/ 407); p=0.0048], as well as the number of false positives [18.9% (77/ 407), *versus* 10.3% (42/407); p=0.0005] (Table 2).

**Table 2: Performance of *Pf*HRP2- based rapid diagnostic test performed by study nurses at health facilities (HF-*Pf*HRP2) or *Pf*HRP2-based rapid diagnostic test performed at the central microbiology laboratory (Lab *Pf*HRP2) by trained technicians compared with expert microscopy (gold standard).**

| Performance characteristic | HF-*Pf*HRP2 n (%) | Lab-*Pf*HRP2 n (%) | p-value |
|---|---|---|---|
| True positive | 238 (58.5) | 240 (59.0) | 0.8848 |
| True negative | 86 (21.1) | 121 (29.7) | 0.0048 |
| False positive | 77 (18.9) | 42 (10.3) | 0.0005 |
| False negative | 06 (1.5) | 04 (1.0) | 0.5209 |

The sensitivity and specificity of the *Pf*HRP2-RDT supplied by the NMCP and performed by the nurses at health facilities level (HF-*Pf*HRP2) was 97.5% and 52.8%, respectively when using expert microscopy as reference (Table 3). The sensitivity and specificity of the *Pf*HRP2 RDT purchased by CRUN and performed by trained laboratory technicians at laboratory level (Lab-*Pf*HRP2) was 98.4% and 74.2%, respectively (using expert microscopy as gold standard). The positive predictive value of the HF-*Pf*HRP2 was 75.6% and 85.1% for the Lab-*Pf*HRP2 (Table 3). The negative predictive value of the HF-*Pf*HRP2 was 93.5% and that of the Lab-*Pf*HRP2 was 96.8% (Table 3).

**Table 3: Diagnostic accuracy of *Pf*HRP2 based rapid diagnostic test performed by study nurses at health facilities (HF-*Pf*HRP2) and *Pf*HRP2 based rapid diagnostic test performed at the central microbiology laboratory (Lab *Pf*HRP2) by trained technicians compared using expert microscopy as gold standard.**

| Diagnostic Performance characteristic | HF-*Pf*HRP2 | | Lab-*Pf*HRP2 | |
|---|---|---|---|---|
| | % (n/N) | 95% CI | % (n/N) | 95% CI |
| Sensitivity | 97.5 (238/244) | 94.7-99.1 | 98.4 (240/244) | 95.9-99.6 |
| Specificity | 52.8 (86/163) | 44.8-60.6 | 74.2 (121/163) | 66.8-80.8 |
| Positive predictive value | 75.6 (238/315) | 72.4-78.5 | 85.1 (240/282) | 81.5-88.1 |
| Negative predictive value | 93.5 (86/92) | 86.5-97.0 | 96.8 (121/125) | 91.9-98.8 |

The agreement between expert microscopy and HF-*Pf*HRP2 was "moderate" (k-value: 0.542), that between expert microscopy and Lab-*Pf*HRP2 was "good" (k-value: 0.755) and that between HF-*Pf*HRP2 and Lab-*Pf*HRP2 also "good" (k-value: 0.707) (Table 4**)**. Only 49.5% (38/77) of malaria false positives found with HF-*Pf*HRP2 were also tested positive with Lab-*Pf*HRP2 (Tables 2 and 5). However, the remaining 50.5% (39/77) of the false positives found with HF-*Pf*HRP2 were tested negative with Lab-*Pf*HRP2 (Table 2 and 5). Furthermore, of the 238 malaria true positive cases reported with HF-*Pf*HRP2, 99.6% (237/238) were also tested positive with Lab-*Pf*HRP2 (Table 2 and 5).

**Table 4: Agreement between the different diagnostic procedures**

| | Number of observed agreement n (%) | Number of agreement expected by change n (%) | Kappa (95% CI) | SE of Kappa | Strength of agreement |
|---|---|---|---|---|---|
| **Field HRP2 and microscopy** | 324 (79.61) | 225.7 (55.45) | 0.542 (0.462-0.623) | 0.041 | Moderate |
| **Lab HRP2 and microscopy** | 361 (88.70) | 219.1 (53.84) | 0.755 (0.690-0.820) | 0.033 | Good |
| **Field HRP2 Lab HRP2** | 360 (88.45) | 246.5 (60.57%) | 0.707 (690-0.820) | 0.039 | Good |

**Table 5: Rapid diagnostic tests results obtained either in the field or in the laboratory compared with expert malaria microscopy findings**

| | Microscopy + N=244 n (%) | | Microscopy – N=163 n (%) | | |
|---|---|---|---|---|---|
| | **HRP2 + (lab)** | **HRP2 - (lab)** | **HRP2 + (lab)** | **HRP2 - (lab)** | **Total** |
| **HRP2 + (field)** | 237 (97.1) | 1 (0.4) | 38 (23.3) | 39 (23.9) | 315 |
| **HRP2 – (field)** | 3 (1.2) | 3 (1.2) | 4 (2.4) | 82 (50.3) | 92 |
| **Total** | 240 | 4 | 42 | 121 | 407 |

## Discussion

Rapid diagnostic tests (RDTs) for malaria are widely implemented by National Malaria Control Programmes (NMCP) in endemic countries, including Burkina Faso, in order to meet the WHO requirement of confirming a malaria infection before starting a treatment (1) (2). RDTs are hence increasingly replacing (expert) microscopy in many settings, but there is a concern about the diagnostic accuracy of HRP2-based RDTs. Several studies reported a lower sensitivity of

RDTs compared to expert microscopy when the parasitaemia is <200 parasites/µl (24). This situation is exacerbated by the fact that *Pf*HRP2 polymorphisms are being reported and that certain deletions in this gene may negatively affect RDT performance (25) (26) (27). These *Pf*HRP2 gene deletions have so far not been found in Burkina Faso, but do occur in neighbouring Mali (25). However, a sensitivity issue was not observed in the present study. There was no significant difference in test sensitivity when the RDT was performed by the two different groups of operators (nurses in the rural health facilities compared to trained laboratory technicians). Importantly, the RDT sensitivity and NPV achieved by both groups almost reached the level of expert microscopy. Only few false negative results were reported with the employed RDTs.

In contrast, the specificity (and subsequently the PPV) of the RDT was worrying low (52.8%) when the test was performed by the nurses at the health facilities level. This is also reflected in the observed agreement between the tests. Overall, the agreement between expert microscopy and Lab-HRP2 was good, but moderate between HF-RDT and expert microscopy. This could be explained by the fact that the HF-RDT was more often false positive. In general, the specificity of the HRP2-based RDTs is being questioned particularly under low transmission conditions (20). This is supported by one of previous studies conducted in the same study area in which a high prevalence of false positive RDT results was reported during the dry season (April-May; low transmission) (22). However, the present study was mainly conducted during the rainy season (June-October; high transmission season) but still the number of false positive tests was almost two-fold higher at the participating health facilities compared to the laboratory of CRUN. It is more obvious that the number of false positive cases could thus be higher if the study was conducted mainly during the dry season.

HRP2 persistence after a successful treatment is often used as an explanation for the lower specificity of RDTs that are based on the detection of this specific antigen (12) (13) (14) (15) (16) (17) (18) (19). This can however not explain the difference in the test performance observed between the two different groups of operators (i.e. health facility nurses' *vs* laboratory technicians). Several other factors can influence the RDT performance including incorrect test execution and reading of RDT results by health facility nurses whilst performing the test (9) (10). Also operator errors such as incorrect application of the blood sample or running buffer on the test device, substituting test kit buffer solution with other liquids such as normal saline, diluted water, tap water or buffer from different kits/lots/batches or faulty test devices can affect the test performance by health facility nurses (11). A very long-reading time could also

explain the high positive rate of false positive at health levels. Some non-specific binding or interaction with other immunological or infection factors such as rheumatoid factors, hepatitis C, schistosomiasis, toxoplasmosis, dengue, leishmaniasis, Chagas' disease and human African trypanosomiasis can lead to a malaria false positive reaction on a HRP2-based RDT, though considered to be rare (6) (28) (29) (30) (31) (32) (33) (34) (35) (36). It is therefore crucial to ensure adequate training of the health workers who perform the RDT and periodically monitor the execution of malaria RDT by the health workers (37).

According to the WHO testing report and the RDT manufacturer information note, the *P. falciparum* specific HRP2-based RDT can stand up to 40°C for 24 months (6). However, in Burkina Faso, the mean maximum temperature can reach 45°C in the dry season (38). The participating health facilities in this study had no air-conditioning system or temperature and humidity monitoring system in their store room. This can severely affect the test performance as previously reported (8) (39) (40). Moreover, periodical quality checks of the RDT at the health facilities are not in place. The above-mentioned issues should all be addressed when implementing the malaria *Pf*HRP2 RDT.

Finally, the blood specimen used to perform the malaria RDTs in the field was a capillary sample and the one used to perform malaria RDTs in the central laboratory was from venous blood. It has been reported that the sensitivity of malaria tests (i.e. microscopy) depend on the site of blood collection, in particular in asymptomatic malaria cases. Capillary blood tends to be more sensitive than venous blood (41). However, the clinical symptoms of malaria infection, including fever, occur in synchrony with the rupture of infected erythrocytes and the release of these erythrocytes and malaria debris in circulating blood (42) (43). So, it is obvious that symptomatic malaria, which was studied in the present research, will be detectable in capillary blood as well as venous blood.

**Conclusion**

Rapid diagnostic tests are a valuable tool for the diagnosis of malaria in settings where expert microscopy is not available. However, some external factors could negatively influence the performance of these RDTs in the field. As long as these factors remain, causes of fever might not be correctly diagnosed and results in inappropriate prescription of anti-malarials and antibiotics in fear of overlooking a treatable infection.

**Abbreviations**

CRUN: Clinical Research Unit of Nanoro

EDTA: Ethylene Diamine Tetra Acetic acid

FIND: Foundation for Innovative New Diagnostics

HF: Health Facility

HRP2: Histidine-Rich Protein

Lab: Laboratory

LoD: Limit of Detection

*Pf*: *Plasmodium falciparum*

NMCP: National Malaria Control Program

NPV: Negative Predictive Value

*p*LDH: *Plasmodium*-specific parasite lactate dehydrogenase

PPV: Positive Predictive Value

RDT: Rapid Diagnostic Test

SD: Standard Diagnostic

SSA: Sub-Sahara Africa

WBC: White Blood Cells

WHO: World Health Organization

**Declarations**

*Ethics approval and consent to participate*

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent for the participation of the children was obtained from parents or legal guardians prior to enrolment in the study.

*Consent for publication*

Not applicable.

*Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors declare that they have no competing interests

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*Authors' contributions*

FK, HS, PM, HT and MBvH conceived and designed the study. FK, MT and MB supervised patient inclusion, signature of informed consent and diagnostic specimen collection by study nurses. KF, MT and MB performed/supervised the laboratory analyses (malaria microscopy and the retest of malaria RDTs). FK analyzed the data under the supervision of a biostatistician. FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript.

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# *Chapter 5*

## *Accuracy of a Plasmodium falciparum specific histidine-rich protein 2 rapid diagnostic test in the context of the presence of non-malaria fevers, prior anti-malarial use and seasonal malaria transmission*

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**Abstract**

**Background** It remains challenging to distinguish malaria from other fever causing infections, as a positive rapid diagnostic test does not always signify a true active malaria infection. This study was designed to determine the influence of other causes of fever, prior anti-malarial treatment, and a possible seasonality of the performance of a *Pf*HRP2 RDT for the diagnosis of malaria in children under-5 years of age living in a malaria endemic area.

**Methods:** A prospective etiology study was conducted in 2015 among febrile children under 5 years of age in Burkina Faso. In order to assess the influence of other febrile illnesses, prior treatment and seasonality on the performance of a *Pf*HRP2 RDT in diagnosing malaria, the RDT results were compared with the gold standard (expert microscopic diagnosis of *Plasmodium falciparum*) and test results were analysed by assuming that prior anti-malarial use and bacterial/viral infection status would have been known prior to testing. To assess bacterial and viral infection status blood, urine and stool samples were analysed.

**Results:** In total 683 blood samples were analysed with microscopy and RDT-*Pf*HRP2. *Plasmodium falciparum* malaria was diagnosed in 49.8% (340/683) by microscopy compared to 69.5% (475/683) by RDT-*Pf*HRP2. The RDT-*Pf*HRP2 reported 29.7% (141/475) false positive results and 1.8% (6/340) false negative cases. The RDT-*Pf*HRP2 had a high sensitivity (98.2%) and negative predictive value (97.1%), but a low specificity (58.9%) and positive predictive value (70.3%). Almost 50% of the alternative cause of fever were diagnosed by laboratory testing in the RDT false positive malaria group.

**Conclusion:** The use of a malaria RDT-*Pf*HRP2 in a malaria endemic area may cause misdiagnosis of the actual cause of fever due to false positive test results. The development of a practical diagnostic tool to screen for other causes of fever in malaria endemic areas is required to save lives.

**Keywords** RDT-*Pf*HRP2, diagnosis, malaria, fever, sensitivity, specificity, accuracy.

**Background**

With the recommendation of the World Health Organization (WHO) to confirm a malaria infection in a febrile child before commencing treatment, malaria rapid diagnostic tests (RDTs) have become indispensable in the screening of malaria-suspected fever cases (1). The main goal of this positive development is to substantially reduce unnecessary prescription of anti-malarials and hence decrease inappropriate treatment (2) (3). However, persisting antigen after adequate treatment or spontaneous remission, which can be detected by the RDT employed, might jeopardize this aim (4). This is most evident in the case of the diagnostic target antigen histidine-rich protein-2 (*Pf*HRP2), which is specific to *Plasmodium falciparum*, and that can persist in the blood for weeks after treatment (5) (6) (7). This antigen persistence could increase the rate of false positive test results, leading to a wrong diagnosis in settings where other diagnostic tools are unavailable. This could in particular be the case when the fever persists or relapses after malaria treatment. If malaria is successfully treated the *Pf*HRP2-based RDT (RDT-*Pf*HRP2) will remain positive, suggesting a malaria infection, whereas the actual cause of fever is due to another infection (2) (3) (8). This phenomenon of false positive results after successful treatment can result in an overestimation of malaria positive cases and lead to misdiagnosis of the true cause of fever in children. Recent studies have reported a decreasing specificity and positive predictive value of the RDT-*Pf*HRP2 up to 3–4 weeks after successful malaria treatment (2) (3) (9) (10). This period of antigen persistence leads to a diagnostic gap in which HRP-2 based tests cannot be used. Although RDT-*Pf*HRP2 allows to treat a large part of children who actually have malaria, the children with false positive results could be suffering from alternative causes of fever like bacterial or viral infections (9).

Up to date several studies have assessed the performance of malaria RDT-*Pf*HRP2 in different malaria transmission areas under controlled conditions. For example, Grandesso et *al*. (5) showed that the sensitivity of two different HRP-2 based tests in a low or high transmission area ranged from 98.4 to 99.2%, with no significant difference between tests and settings. However, the specificity of the HRP-2 based tests was much lower in high transmission settings (79.7–80.7%) compared to the low transmission settings (98.8–98.8%). In general, the specificity of HRP-2 based RDTs seems to decrease with an increase in *P. falciparum* malaria (11). This specificity issue of the HRP2 RDT may thus affect its usefulness in health care practice. However, limited data are available on the influence of other causes of fever on the performance of this RDT in rural areas where mainly febrile children attending the health facilities are being screened with malaria RDTs to determine the cause of their fever. If the

performance of the RDT-*Pf*HRP2 is affected by other infections in malaria endemic areas, the end result might be that other potential causes of fever are being ignored, and that the fever is still being treated as if it is malaria.

In Burkina Faso, like many other malaria endemic areas, the introduction of malaria RDT-*Pf*HRP2 significantly contributed to reducing anti-malarial prescriptions, mainly in children (12). However, as an unwanted effect it could increase the untargeted use of antibiotics to treat fever (13). Previous studies in Burkina Faso have reported on the performance of malaria RDT-*Pf*HRP2 tests in relation to the accuracy of a RDT for the diagnosis of both malaria and malaria-attributable fever or to other infections (14) (15). However, there are no studies from Burkina Faso assessing the number of wrongly diagnosed cases with other febrile diseases when only the results of the RDTs are used for patient management. Therefore, the present study was designed to determine the influence of other causes of fever and previous malaria infections on the performance of RDT-*Pf*HRP2 for the diagnosis of malaria in children under-5 years living in a malaria endemic area.

## Methods

### Study site

The study was conducted in the health district of Nanoro, located in central-west part of Burkina Faso. Nanoro is around 100 km from Ouagadougou, the capital of the country. The data were collected in four peripheral health facilities of the health district of Nanoro (i.e. Nanoro, Godo, Nazoanga and Seguedin) and the referral hospital Saint Camille of Nanoro. These peripheral health facilities have been chosen as study locations because of their accessibility and close distance (the most remote is 20 km) from the central laboratory. CMA (Centre Medical avec Antenne Churigicale) Saint Camille of Nanoro was included to increase inclusion of severe cases that were referred from peripheral health facilities. The district hospital CMA Saint Camille de Nanoro is the reference hospital of the health district of Nanoro with trained medical staff and equipped laboratory facilities for the management of difficult medical cases that are referred from the peripheral health facilities. The peripheral health facilities are the first point of medical contact within the community for the management of less complicated medical cases by Community Health Workers based on guideline of diseases management (16). Malaria is the first cause of consultation in children under-5 years of age in this region and predominately occurs during the rainy season July-November.

**Study design**

This study has been conducted as part of a larger project that aims to improve the diagnosis and management of non-malaria fevers in children under-5 years of age in Nanoro, Burkina Faso. Briefly, all children under-5 years of age documented with axillary temperature ≥37.5°C presenting at the participating health facilities were invited to participate in the study. Written informed consent was obtained from parent/guardian before any data collection. All participants were tested systematically for malaria infection with RDT and managed according to the test result as per National Malaria Control Programme (NMCP) guidelines. In addition, expert malaria microscopy was performed for the purpose of studying the fever aetiology and the accuracy of the RDT-*Pf*HRP2 employed. Furthermore, clinical specimens like blood and urine for bacterial cultures, and stool for rotavirus and adenovirus tests were also collected systematically and analysed at the microbiology laboratory of the Clinical Research Unit of Nanoro (CRUN). This study was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130).

**Laboratory procedure**

*Malaria rapid diagnostic test*

The malaria Rapid Diagnostic Test recommended by the Burkinabe NMCP is the SD Ag Bioline *Pf* (Standard Diagnostics, Hagal-Dong, Korea) detecting *Pf*HRP2 and this test was performed by trained study nurses at the health facilities or the district hospital. The nurses recorded malaria RDT results as positive or negative on study case record forms. During the last WHO-FIND testing round 6 the specificity and sensitivity for this particular test was reported to be high (>95%) (17).

*Microscopy*

Malaria diagnosis by microscopy was performed by expert laboratory technicians at CRUN. These expert microscopists are frequently submitted to an external quality control programme and only certified microscopists are allowed to read the slides. The LoD of this expert microscopy 10 parasites/µl. Thick and thin blood smears were prepared (in duplicate) from blood collected in the Ethylene Diamine Tetra Acetic acid (EDTA) tubes. Thin films were fixed with methanol and blood slides were stained with 3% Giemsa solution (pH 7.2) for identification and quantification of asexual *P. falciparum* and others *Plasmodium* species. Parasites densities were determined by counting the number of asexual parasites per 200 white blood cells, and calculating per µl of blood by assuming the number of white blood cells to be

at 8,000/µl. Thick blood smears were considered negative when the examination of 100 fields per thick film did not reveal the presence of any asexual parasites. Each blood slide was read by two independent expert readers, and in case of discordance (positive *vs* negative, different in *Plasmodium* species, difference in parasite density >Log10 or ratio>2 in case of parasite density ≤400/µl or >400/µl, respectively) by a third independent reader whose conclusion was decisive. Positive microscopy results were recorded as the geometric means of the two reader's results or the geometric means of the two geometrically closest reading in case of third reading. These results were expressed as asexual parasites per microliter by using the patient's white blood cell (WBC) count. A selection of slides (5%) was re-read by an independent expert microscopist for quality assurance.

*Microbiology*

For blood cultures, 1–3 ml of venous blood was collected into a paediatric blood culture bottle (BD BACTEC Peds Plus™/F, Becton Dickinson and Company, Sparks, Maryland, USA) and incubated in a BACTEC 9050 instrument (Becton Dickinson) for a total of 5 days according to the manufacturers' protocol. If flagged for growth the cultures were Gram stained, sub-cultured on Eosin-Methylene Blue (EMB) agar, 5% Sheep Blood agar (bioMérieux, Marcy-l'Etoile, France) and chocolate + isoVitalex agar, and incubated at 35–37°C for 24 hours in atmospheric condition for EMB and at $CO_2$ for Sheep Blood agar and chocolate + isoVitalex agar. Isolates were identified by standard microbiological methods like described in Mackie and McCartney Practical Medical Microbiology (18) and biochemical test (API strips, bioMerieux Marcy-l'Etoile, France).

Urine culture was done when a urine sample was positive for leucocytes and nitrite as indicated by a urine dipstick (Standard Diagnostics, UroColor, Inc, Korea). Urine was inoculated on CLED (Cystine Lactose Electrolyte Deficient) and EMB agar. The cultures were incubated at 37°C during 24 hours. Only samples that generated pure bacterial growth of more than $10^5$ colonies forming units (CFU)/ml were regarded as yielding significant bacteriuria. A count of $\leq 10^5$ CFU/ml was regarded as negative. Mixed growths (growth of more than one species in a sample, in particular growth of normal skin flora picked up during urine collection) were regarded as contaminated and therefore disregarded. A second urine sample was not collected. Pathogen identification was done by standard microbiology biochemical methods (API system, bioMerieux Marcy-l'Etoile, France).

Stool samples were specifically analysed for group A rotavirus using one step rotavirus and adenovirus serotype 40/41 in human feces test (SD Bioline Rota/Adeno; Standard Diagnostic, Inc., Korea) as these pathogens are considered to cause fever.

For the purpose of this study bacterial bloodstream infections (bBSI), viral gastro-intestinal infection (vGII) caused by rotavirus and adenovirus, and urinary tract infections (UTI) were considered as alternative cause of fever, different from malaria (19) (20) (21) (22).

**Data analysis**

Double entry of data was performed by two different persons by using OpenClinica software. The data analysis was done by using STATA 13. Description of qualitative and quantitative variables were performed by using proportion and mean or median, respectively. The geometric mean was used to express the parasite densities. The performance of RDT was evaluated compared to microscopy by calculating the sensitivity, the specificity, negative and positive predictive value of the RDT.

To assess the influence of alternative causes of fever as defined for the purpose of this study and prior use of anti-malarials on the RDT performance, it was assumed that infection status and prior anti-malarial use was known before RDT testing and, therefore, a correction was done by removing all confirmed cases of bloodstream infections, urinary tract infections, viral gastro-intestinal infections and prior use of anti-malarials within 2 weeks from the analysis either separately or together.

The rainy season was from July to November and dry season from December to June, and an analysis, done by season next to a whole year, was done on the results The Cohen's Kappa coefficient was computed to assess to agreement between microscopy and RDT. $p \leq 0.05$ was considered statistical significant.

**Results**

**General study characteristics**

In total 1447 children under-5 years of age attending the peripheral health facilities or the CMA Saint Camille of Nanoro between January to December 2015 were screened. Of these children, 684 children under-5 years with axillary temperature ≥37.5°C at presentation were enrolled in the study. One child was excluded at a later stage because the malaria RDT was not performed at enrolment. The baseline characteristics of the remaining 683 febrile children are summarized in Table 1.

**Table 1: Baseline characteristics of the study population enrolled at the health facilities and the district hospital**

| | Included N=683 | Malaria microscopy positive n=340 | Malaria microscopy negative n=343 | *Pf*HRP2 positive n=475 | *Pf*HRP2 negative n=208 |
|---|---|---|---|---|---|
| **Sex** (%) | | | | | |
| Male | 369 (54.0) | 176 (51.8) | 193 (56.3) | 256 (53.9) | 113 (54.3) |
| Female | 314 (46.0) | 164 (48.2) | 150 (43.7) | 219 (46.1) | 95 (45.7) |
| **Age** (%) | | | | | |
| ≤12 months | 200 (29.3) | 64 (18.8) | 136 (39.7) | 110 (23.2) | 90 (43.3) |
| >12 months | 483 (70.7) | 276 (81.2) | 207 (60.3) | 365 (76.8) | 118 (56.7) |
| **Temperature** (%) | | | | | |
| ≥37.5°C-≤38.5°C | 340 (49.8) | 153 (45.0) | 187 (54.5) | 221 (46.5) | 119 (57.2) |
| >38.5°C-≤39.5°C | 241 (35.3) | 123 (36.2) | 118 (34.4) | 177 (37.3) | 64 (30.8) |
| >39.5°C | 102 (14.9) | 64 (18.8) | 38 (11.1) | 77 (16.2) | 25 (12.0) |
| All causes (%) | 73 (10.7) | 11 (3.2) | 62 (18.1) | 47 (9.9) | 26 (12.5) |
| BSI (%) | 41 (6.0) | 6 (1.8) | 35 (10.2) | 29 (6.1) | 12 (5.8) |
| UTI (%) | 10 (1.5) | 3 (0.9) | 7 (2.0) | 9 (1.9) | 1 (0.5) |
| Rotavirus/Adenovirus (%) | 25 (3.7) | 3 (0.9) | 22 (6.4) | 12 (2.5) | 12 (5.8) |

BSI: Bloodstream infection; UTI: urinary tract infection; *Pf*HRP2: *Plasmodium falciparum* histidine–rich protein-2.

### Performance of RDT compared to microscopy and malaria treatment

The diagnostic performance of the malaria RDT-*Pf*HRP2 is reported in Table 2. *Plasmodium falciparum* infection was diagnosed by microscopy in 49.8% (340/683) and by RDT-*Pf*HRP2 in 69.5% (475/683) of the febrile children. The proportion of fever attributable to malaria as determined by expert microscopy was lower in the dry season (29.1%; 100/344) than in the rainy season (70.8%; 240/339). Out of the 475 febrile children with a positive RDT result, 29.7% (141/475) were found negative by expert malaria microscopy and thus considered RDT false positive. Stratifying for seasonality showed that RDT false positives were more prevalent during the dry season (46.4%; 84/181) compared to the rainy season (19.4%; 57/294). There were 2.9% (6/208) false negative cases; i.e. children positive by microscopy but negative by RDT.

**Table 2: Agreement between expert malaria microscopy and *Pf*HRP2 RDT**

| | True positive n (%) | True negative n (%) | False positive n (%) | False negative n (%) |
|---|---|---|---|---|
| **Whole year (N=683)** | 334 (48.9) | 202 (29.6) | 141 (20.6) | 6 (0.9) |
| **Dry season (N=344)** | 97(28.2) | 160(46.5) | 84(24.4) | 3(0.9) |
| **Rainy season (N=339)** | 237(69.9) | 42(12.4) | 57(16.8) | 3(0.9) |

Number of observed agreements:536 (78.48% of the of the observation)
Number of agreement expected by chance: 340.9 (49.91% of the observation)
Kappa= 0.570; p-value<0.00001
SE of Kappa= 0.029
95% confidence interval: From 0.514 to 0.627
The strength of agreement is considered to be "moderate".

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the RDT-*Pf*HRP2 were 98.2% (334/340), 58.9% (202/343), 70.3% (334/475) and

97.1% (202/208), respectively (see Table 3). The agreement between microscopy and RDT was moderate with a Cohen's Kappa coefficient of 0.57 ($p<0.00001$). Interestingly the *Pf*HRP2 RDT showed to be more specific (65.6%; 160/244) in the dry season than during the rainy season (42.9%; forty-two of 99) (see Table 3).

**Table 3: Diagnostic performance of *Pf*HRP2-RDT compared with expert microscopy (gold standard) for detection of malaria in febrile children**

| Accuracy parameters | Value % (n/N) | Confidence intervals (95% CI) |
|---|---|---|
| **Diagnostic performance all year** | | |
| Sensitivity | 98.2 (334/340) | 96.2 - 99.3 |
| Specificity | 58.9 (202/343) | 53.5 - 64.1 |
| Positive predictive value | 70.3 (334/475) | 66 - 74.4 |
| Negative predictive value | 97.1 (202/208) | 93.8 - 98.9 |
| **Diagnostic performance during dry season** | | |
| Sensitivity | 97.0 (97/100) | 91.5 - 99.4 |
| Specificity | 65.6 (160/244) | 59.2 - 71.5 |
| Positive predictive value | 53.6 (97/181) | 46 – 61 |
| Negative predictive value | 98.2 (160/163) | 94.7 - 99.6 |
| **Diagnostic performance during rainy season** | | |
| Sensitivity | 98.8 (237/240) | 96.4 - 99.7 |
| Specificity | 42.4 (42/99) | 32.5 - 52.8 |
| Positive predictive value | 80.6 (237/294) | 75.6 – 85 |
| Negative predictive value | 93.3 (42/45) | 81.7 - 98.6 |

**Other causes of fever and influence of previous malaria treatment on RDT performance**

In the RDT malaria false positive group, 25.5% (36/141) of the children were found to have other causes of fever based on the laboratory findings (Table 4). The most common was a bacterial bloodstream infection (bBSI; 16.3%; 23/141), followed by rotavirus and adenovirus infections (vGII; 6.4%; 9/141) and urinary tract infection (UTI; 4.3%; 6/141). For the alternative causes of fever, 49.3% (36/73) were diagnosed in malaria RDT-*Pf*HRP2 false positive group, 35.6% (26/73) in malaria RDT-*Pf*HRP2 true negative and 15.1% (11/73) in malaria RDT-*Pf*HRP2 true positive group. The difference of alternative causes of fever between malaria RDT-PfHRP2 false positive group 25.53% (36/141) and malaria RDT-*Pf*HRP2 true negative group 12.38% (26/202) was statistically significant ($p=0.002$). It was also observed that there was no significant difference ($p=0.632$) in geometric mean parasite densities between the true RDT malaria positive children either with (26279.52: 211349.7–32347.7) or without (23957.4: 19378.7–29617.9) an additional cause of fever.

In the true (by microscopy) malaria negative group, 12.9% (26/202) of the children were infected by pathogens that are known to cause fever and that were assessed in this study. Of the children that were confirmed to be positive for malaria by microscopy, only 3.3% (11/334) were co-infected with other causes of fever that were considered in this study (Table 4). During

the data collection, 5.0% (31/683) parents or guardians declared to have given anti-malarial medication to her/his child within 2 weeks before inclusion. Among these children there were 17 cases in the true malaria negative group and 13 cases in the false malaria positive group. One child in the true malaria positive group declared to have taken anti-malarial within 2 weeks before inclusion.

**Table 4: Laboratory findings and previous antimalarial use reported per RDT outcome**

| Characteristics | n/N (%) |
|---|---|
| **False malaria positive** | 141 |
| Laboratory data | |
| Alternative cause of fever | 36/141 (25.5) |
| Bacterial bloodstream infection (BSI) | 23/141 (16.3) |
| Rotavirus/Adenovirus | 9/141 (6.4) |
| Urinary tract infection (UTI) | 6/141 (4.3) |
| Previous antimalarial use reported | 14/141 (9.9) |
| **True malaria negative** | 202 |
| Laboratory data | |
| Alternative cause of fever | 26/202 (12.9) |
| Bloodstream infection (BSI) | 12/202 (5.9) |
| Rotavirus/Adenovirus | 13/202 (6.4) |
| Urinary tract infection (UTI) | 01/202 (0.5) |
| Previous antimalarial use reported | 19/202 (9.4) |
| **True malaria positive** | 334 |
| Laboratory data | |
| Alternative cause of fever | 11/334 (3.3) |
| Bloodstream infection (BSI) | 06/334 (1.8) |
| Rotavirus/Adenovirus | 03/334 (0.9) |
| Urinary tract infection (UTI) | 03/334 (0.9) |
| Previous antimalarial use reported | 01/334 (0.3) |

**Correction of RDT performance by using outcomes of other diagnostic testing**

One of the aims of the study was to assess the sensitivity and specificity of the employed RDT if it would be possible to exclude patients with prior anti-malarial use and bacterial infections. The corrected performance of the RDT is reported in Table 5 and the corrected accuracy in Table 6. In that case, the overall specificity of the RDT would have increased from 58.9% (202/343) to 63.0% (162/257) and the PPV from 70.3% (334/475) to 77.1% (320/415) (Table 6). The sensitivity (98.2%; 334/340) would not change and the NPV would have decreased slightly from 97.1% (202/208) to 96.4%). The agreement after correction between the microscopy and the RDT was moderate with a Cohen's Kappa coefficient of 0.62 ($p<0.001$). This effect would be larger in the rainy season as the specificity would have increased from 42.4% (42/99) to 50.7% (35/69) and the PPV from 80.6% (237/294) to 87.2% (231/265). The effect would have been less marked in the dry season where the specificity would have increased from 65.6% (160/244) to 67.6% (127/188) and the PPV from 53.6% (97/181) to

59.3% (89/150). Taking the result of bBSI into account had the largest effect on RDT performance and prior anti-malarial intake had the smallest effect.

**Table 5: Diagnostic performance of the RDT after correcting for the influence of other febrile illnesses and previous antimalarial intake per season.**

| | True positive n (%) | True negative n (%) | False positive n (%) | False negative n (%) |
|---|---|---|---|---|
| **Performance if bBSI, UTI, vGII and previous antimalarial intake are excluded** | | | | |
| **Whole season (N=583)** | 320 (54.9) | 162 (27.8) | 95 (16.3) | 6 (1.0) |
| **Dry season (N=280)** | 89 (31.8) | 127 (45.4) | 61(21.8) | 3(1.1) |
| **Rainy season (N=303)** | 231 (76.2) | 35 (11.5) | 34(11.2) | 3(1.0) |
| **Performance after exclusion of bBSI** | | | | |
| **Whole season (N=642)** | 328(51.1) | 190(29.6) | 118(18.4) | 6(0.9) |
| **Dry season (N=326)** | 91(27.9) | 153(46.9) | 79(24.2) | 3(0.9) |
| **Rainy season (N=316)** | 237(75.0) | 37(11.7) | 39(12.3) | 3(0.9) |
| **Performance after exclusion of UTI** | | | | |
| **Whole season (N=671)** | 329(49.0) | 201(30.0) | 135(20.1) | 6(0.9) |
| **Dry season (N=342)** | 97(28.4) | 159(46.5) | 83(24.3) | 3(0.9) |
| **Rainy season (N=329)** | 232(70.5) | 42(12.8) | 52(15.8) | 3(0.9) |
| **Performance after exclusion of vGII** | | | | |
| **Whole season (N=647)** | 326(50.4) | 188(29.1) | 127(19.6) | 6(0.9) |
| **Dry season (N=318)** | 94(29.6) | 147(46.2) | 74(23.3) | 3(0.9) |
| **Rainy season (N=329)** | 232(70.5) | 41(12.5) | 53(16.1) | 3(0.9) |
| **Performance if UTI and vGII are excluded** | | | | |
| **Whole season (N=615)** | 325(52.8) | 171(27.8) | 113(18.4) | 6(1.0) |
| **Dry season (N=295)** | 94(31.9) | 133(45.1) | 65(22.0) | 3(1.0) |
| **Rainy season (N=320)** | 231(72.2) | 38(11.9) | 48(15.0) | 3(0.9) |
| **Performance if antimalarial intake in the previous 2 weeks is excluded** | | | | |
| **Whole season (N=648)** | 333(51.4) | 182(28.1) | 127(19.6) | 6(0.9) |
| **Dry season (N=318)** | 97(30.5) | 143(45.0) | 75(23.6) | 3(0.9) |
| **Rainy season (N=330)** | 236(71.5) | 39(11.8) | 52(15.8) | 3(0.9) |

Number of observed agreements:499 (81.80% of the of the observation)
Number of agreement expected by chance: 314.7 (51.59% of the observation)
Kappa= 0.624; p-value<0.00001
SE of Kappa= 0.030
95% confidence interval: From 0.565 to 0.684
The strength of agreement is considered to be "good".

## Discussion

This study showed that a large proportion of children treated for malaria based on a positive HRP2 RDT results were children who were not infected with malaria if the result of expert microscopy is considered as gold standard. Around 25% of these children actually had another treatable bacterial infection that is missed if only the result of the HRP2 RDT for malaria was followed for treatment. Adding a test for bacterial infections and the history of previous malaria treatment somewhat increased RDT test performance in terms of specificity and PPV. This may subsequently reduce malaria overtreatment. Interestingly seasonality had an influence on RDT performance as well as the contribution of other diseases that were assessed in this study on RDT performance. Possibly, the intensity of malaria transmission, which is different between the dry and the rainy season in the study area (Nanoro), could explain the influence of the seasonality on the performance of the RDT-*Pf*HRP2 as also suggested (14).

The most important finding of the present study is thus the relative high number of malaria RDT false positive results found, if expert malaria microscopy is considered as golden standard. The number found in the present study is in line with those reported by Maltha *et al*. and Tinto *et al*. in the same study area (15) (23) and several studies in other malaria endemic areas (24) (25). In particular, it is noted that Malta et *al*. reported that 27% of the positive *Pf*HRP2 cases did not have an actual malaria infection and that *Pf*HRP2 is less specific during rainy season compared to dry season, which is in line with the study of Bisoffi *et al*. (14) and confirmed in the present study.

One of the generally accepted reasons for false positive RDT results is persisting antigen after adequate treatment or spontaneous remission (11). However, in the present study the contribution of previous treatment seems to be little, as only 5% of the parents have reported previous anti-malarial treatment. Other factors that could have contributed to the observed false positivity of the RDT can be as follows. Firstly, the sensitivity of microscopy may be below that of RDTs. It has for example been shown by Bisoffi *et al.* (14) that the RDT employed in that study had a LoD of 50 parasites/µl and that some of the false positive RDT results are in fact false negative microscopy results. However, the microscopists in the current study are submitted to an external control programme and only accredited microscopists are allowed to read malaria thick blood smears (23). The LoD of this expert microscopy is around 10 parasites/µl and of the employed RTD (recommended by the NMCP of Burkina Faso) is also around 50 parasites/µl as reported by Tinto *et al.* (26). The lowest parasite density observed in our study was 72 parasites/µl. Therefore it is not very likely that false negative microscopy has attributed much to false positive RDTs observed in the present study. Future studies should take this point into consideration and may want to establish the LoD of the employed RDT in advance.

Secondly, the RDT-*Pf*HRP2 used in the present study was ordered in the framework of the implemented programme for the management of malaria in the health district. The research team did not have any control over the transportation and storage conditions of the tests and there is a certain level of uncertainty if these might have affected test performance. Albertini *et al.* (27) showed that transport and storage conditions of malaria RDTs often exceed recommended temperatures and this may affect test performance. However, the tests were always handled according to the NMCP guidelines in place and expired tests were never used. Thirdly, the study nurses were not specifically trained in performing the malaria RDT for the purpose of the study. They performed the test according to their routine practice. Possibly a

refresher training would have been appropriate. Whether this explains the higher number of false positives is questionable as health workers sometimes tend to ignore a weak positive line (thus scoring the test as "negative") rather than considering a negative test as being positive (23) (24) (25).

It should be noted that this study was designed to assess the performance of the RDT under actual field conditions and therefore the mentioned limitations are in fact a reflection of the real-life situation. The observation remains that the overestimation of malaria infections by *Pf*HRP2 is a real concern as found in this study and also reported in several studies (15) (23) (24) (25) and needs to be taken into consideration in the management of febrile cases, in particular not to overlook the real cause of fever.

The justification to choose for the implementation of a malaria RDT-*Pf*HRP2 in many malaria endemic countries is based on test performance as reported by WHO panel studies for this RDT (17).The WHO recommends selecting a RDT based on its sensitivity and specificity (which both should be ≥95% (28). The present study only focused on the use of one particular brand of RDT, which might be seen as a limitation of the current study. However, the employed RDT is implemented by the NMCP of Burkina Faso and, therefore, a diagnostic evaluation is warranted. The employed RDT has a very high sensitivity and NPV as confirmed in the present study. These characteristics enables the identification of the majority of malaria positive cases by RDT (98.24%) and these cases have been treated accordingly, in line with WHO and NMCP guidelines. In addition, its excellent NPV ensures that an important part of the true malaria negative cases (97.12%) are correctly diagnosed as negative by RDT-*Pf*HRP2 and it emphasizes the need to further assess the true aetiology of the (non-malaria) fever in the sick child with a specific attention to other infections. In the routine practice of CRUN, patients will first be asked for symptoms and submitted to clinical examination prior to doing a RDT and obviously if they have clear symptoms of pneumonia or urinary or gastro-intestinal infections these will be treated too even if a RDT is positive for malaria.

It is important to note that the low specificity and PPV found in this study can negatively affect the clinical management of febrile cases. Almost 30% of the malaria RDT-*Pf*HRP2 positives cases were in fact malaria negative, as determined by microscopy, and these children received, in line with the NMCP and WHO malaria treatment guidelines, malaria treatment as their test was positive, but unnecessary (hence over prescription of anti-malarials), as they actually do not have the disease (29) and most likely should have been treated with appropriate antibiotics as an alternative to treat the cause of fever.

Moreover, the poor PPV resulted in the misdiagnosis of the actual cause of febrile illness in around 30% of the children that were tested positive by RDT testing, but actually had another disease that caused their fever. Importantly, almost 50% of the alternative cause of fevers found in this study were actually diagnosed in the children that had false positive results with the RDT-*Pf*HRP2. This could leave clinicians and health workers with a huge dilemma in the case of a malaria positive RDT, i.e. either providing immediately anti-malarials or first further investigate other possible causes of fever. In a recent study a proposition of performing a two-step RDT with *Pf*HRP2 and *Plasmodium* Lactate Dehydrogenase (pLDH) was made to improve the specificity *P. falciparum* diagnosis in high transmission settings with little loss of sensitivity (30). HRP2 positive/pLDH negative cases where further studied by microscopy as a confirmative test. Therefore, it is now proposed that additional testing with a urine dipstick for UTI and a faeces dipstick for vGII can further strengthen this algorithm and will further increase the diagnostic performance and hence the management of febrile cases. A prospective study to further validate such an algorithm in routine practice is needed.

It is also essential to emphasize that clinicians and health workers must be aware that if after anti-malaria treatment with adequate drugs the fever does not subside alternative causes of fever must be considered. In the present study it was found that 3.29% of the children that had a confirmed malaria infection, also had another infection that causes fever and these also need to be adequately treated.

Prior anti-malarial treatment mainly concerned principally RDT false positive (and thus microscopy negative) and true negative cases. This finding shows that the anti-malarials prescribed in the study region (principally artemisinin-based combination therapy for uncomplicated malaria) are highly efficient. Therefore, RDT positive cases with prior anti-malarial treatment (<3 weeks) are likely to be false positive, and clinicians and health works should thus be aware of this phenomenon and always thoroughly ask parents or guardians about previous intake use of drugs (4).

The risk of having incorrect results by microscopy is in the present study very low. The microscopists who took part in the present study are submitted to regular certification programmes to ensure the quality of the malaria slide reading performed. Therefore, the persistence of HRP2 antigens detectable up to 3 weeks after successful anti-malarial treatment is considered as an important cause of the false positives (4).

The present study found only few false negative cases (2.88%; 6/208); i.e. children positive by microscopy but negative by RDT. There is increasing concern that *P. falciparum* parasites having deletion(s) in the HRP2 gene yield false negative RDT results (31) (32). This phenomenon has not been studied in Nanaro, but will require attention in future studies as HRP2-based tests are recommended by the NMCP.

**Conclusion**

In conclusion, RDTs based on *Pf*HRP2 have a good sensitivity and negative predictive value, but their use in malaria endemic areas may result in missing true causes of fever mainly in the malaria false positive group. This puts a dilemma to clinicians and health workers that could be circumvented by using a second malaria RDT based on a different target, such as pLDH and/or using other simple diagnostic tests like urine and feces dipstick. In addition, the development of practical tool to screen the other causes of fever in malaria endemic area, should contribute to save live, but also to improve the performance of the malaria RDT view the few cases of co-infection of malaria with these other causes of fever.

**List of abbreviations**

bBSI: bacterial bloodstream infections

CLED: cystine lactose electrolyte deficient

CFU: colonies forming units

CMA: centre medical avec antenne churigicale

CRUN: Clinical research unit of Nanoro

EMB: Eosin-Methylene Blue

NMCP: National Malaria Control Programme

NPV: negative predictive value

*Pf*HRP2: *Plasmodium falciparum* histidine–rich protein-2

RDT-*Pf*HRP2: *Pf*HRP2-based RDT

PCR: polymerase chain reaction

PPV: positive predictive value

RDT: rapid diagnostic test

SD: standard diagnostic

UTI: urinary tract infections

vGII: viral gastro-intestinal infection

WBC: white blood cell

WHO: World Health Organization

**Declarations**

**Ethics approval and consent to participate**

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent was obtained from parents or guardians for the participation of the children prior to enrolment in the study.

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

FK, HS, PM, HT and MBvH conceived and designed the study. FK, MB and MT supervised patient inclusion, taking of informed consent and diagnostic specimen collection by study nurses. KF, PL, MT and MB performed the laboratory analyses. FK and TR analyzed the data. FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript

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# Chapter 6

## *The effect of malaria rapid diagnostic tests results on antimicrobial prescription practices of healthcare workers in Burkina Faso*

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**Abstract**

**Background:** Malaria rapid diagnostic tests (RDT) are widely used in endemic areas in order to comply with the recommendation that malaria treatment should only be given after the clinical diagnosis has been confirmed by RDT or microscopy. However, the overestimation of malaria infection with the use of *Pf*HRP2 based RDT, makes the management of febrile illnesses more challenging. This study aimed to assess the effect of the use of malaria RDT on antimicrobial prescription practices.

**Methods:** A prospective study was conducted among febrile children under-5 years of age attending four health facilities and the referral hospital in the Nanoro Health District (Burkina Faso). To assess the effect of malaria RDT testing on the prescriptions of antimicrobials in febrile children, the initial diagnosis and antimicrobial prescriptions following a malaria RDT testing were recorded. The necessity of these prescriptions was subsequently checked by assessing the actual cause of fever by expert malaria microscopy and a microbiology analysis of blood, urine, stool and nasopharynx swabs that were collected from febrile cases to determine the actual cause of the fever episode.

**Results:** Malaria was diagnosed by nurses, who are the primary health care providers, with a malaria RDT in 72.7% (798/1098) of febrile children, but only 53.7% (589/1097) cases could be confirmed by expert microscopy. Health care workers were likely to prescribe antimalarials to malaria positive RDT compared to malaria negative RDT (RR=7.74, p=0.00001). Malaria negative RDT result had a significant influence on the antibiotic prescriptions (RR=3.57, p=0.0001). The risk of prescribing antimicrobials was higher in health facility level compared to referral hospital. By cross-checking of laboratory findings to antimicrobial prescriptions, an important part of children with positive bacterial infection have received antibiotic prescriptions although the majority without any infection have also received antibiotics.

**Conclusion**: Despite the good attitude of health care workers to adhere to diagnostic test results, antimalarials and antibiotics remain inappropriate prescribed to febrile children. The low specificity of malaria RDT used could be an important cause of these practices.

**Key words**: Prescription, Antimicrobial, Antibiotic, Fever, Malaria, Bacteria and parasites.

**Background**

Acute febrile illnesses in children are globally one of the most common reasons to seek medical care and are associated with considerable morbidity and mortality (1) (2). However, as these conditions often have no specific symptom(s), accurate diagnosis is difficult without laboratory facilities, which is commonly the case in many sub-Saharan countries (SSA) (2) (3) (4) (5) (6). In the past, most febrile episodes encountered in these settings, were considered to be caused by malaria and were treated empirically with antimalarial drugs.

However, the recommendation by the World Health Organization (WHO) to confirm a malaria infection prior to treatment has resulted in the introduction of rapid diagnostic tests (RDT) and in a more focused prescription of antimalarial drugs (7) (8) (9) (10). Nevertheless there are nowadays indications that this positive development in the fight against malaria also has a downside due to an increased use of antibiotics in the malaria RDT negative population and this may contribute to the development of drug resistance (11) (11) (12). RDT's to detect bacterial infections are not readily available in SSA and febrile patients have antibiotics prescribed almost at random in fear of missing a potentially life threatening infection. This situation becomes further complicated due to the fact that the most commonly used malaria RDT, based on *Plasmodium falciparum* histidine-rich protein-2 (*Pf*HRP2), have a specificity problem and may result in a significant number of false positive test results when compared with the golden standard, expert microscopy (13) (14) (15) (16). Consequently, this leads to inappropriate treatment with antimalarials too and the net result of the change from 'presumptive' to 'RDT-*Pf*HRP2 based' malaria management may not be the reduction in malaria over treatment as expected (11) (12) (17).

Moreover, the overestimation of malaria infection due to the use of *Pf*HRP2 based tests makes the management of febrile illnesses more challenging as the actual cause of fever could be missed. A previous study conducted in the same research area reported that probable alternative causes of fever were more prevalent in malaria RDT-*Pf*HRP2 false positive cases (18). The screen and treat strategy for malaria might thus have created a diagnostic dilemma of "non-malaria infections" and how to manage them. Malaria RDT negative cases are treated with antibiotics regardless of the etiology of infection and all malaria false positive cases receive antimalarials unneeded. Consequently, the inappropriate use of antimalarials observed before the introduction of malaria RDT has now been replaced by an increased use of antibiotics in order to not miss potential treatable bacterial infections and of antimalarials due to false positive RDT.

In Burkina Faso, like in many other malaria-endemic areas, HRP2-based RDT is the test authorized by the Ministry of Health based on the specificity and sensitivity (>95%) reported during the last WHO-FIND (World Health Organization-Foundation for Initiative New Diagnostics) to detect *Plasmodium falciparum* malaria infections (19). However, the impact of the introduction of these RDT in clinical practice on the necessity of prescribing antimicrobials (antibiotics, antiparasitics and antimalarials) has not been evaluated. Therefore, in the present study this assessment was performed with the aim to get a better insight into the prescription practices in relation to malaria RDT test results that are available for the clinical staff that manage febrile patients.

**Materials and method**

**Study site**

This study was carried out in the health district of Nanoro, a rural area located around 85 km from Ouagadougou, the capital of Burkina Faso. Data collection was performed at four rural health facilities and the referral hospital (CMA Saint Camille de Nanoro) of Nanoro health district (18).

The peripheral health facilities are the first point of contact for guideline–based management of less complicated medical problems by nurses and for referral of more complicated cases (20). Due to shortage of doctors in Burkina Faso, medical doctors are not available at this first level of the health care system. The referral hospital has medical doctors and well equipped laboratory facilities. Malaria is the first cause of consultation in children under-5 years of age in this region with a high transmission period during the rainy season between July and November (21).

**Study design and enrollment procedure**

To investigate the effect of the use of a malaria RDT on the prescription of antimicrobials in the study area, a detailed descriptive study with a diagnostic component was performed.

The present study was conducted in the framework of a large survey to study the etiologies of fever episodes in children under-5 years of age in the Nanoro Health District (18) (22). The whole population served by the 4 health centres is approximately 37.000 people and children under-5 years represent around 15% of this population (= around 5000 children). We assume that about half of these children (2500 children) will attend one of the health facilities because of fever related health problems. In order to have a representative sample of this population, we aimed to have around 1250 children included in this study (recruited over 1 year). Briefly,

all children under-5 years of age with an axillary temperature ≥37.5°C presenting at one of the participating health facilities or the referral hospital from January to December 2015 and from April to October 2016 were invited to participate in the study. Written informed consent was obtained from parents or legal guardians prior to any data or sample collection. A standard Case Record Form (CRF) was used to collect details on medical history (including prior drug prescription for current fever episodes) and clinical examination. Only the initial antimicrobial prescription made by the health care workers at the first contact with the participants was recorded. All participants were tested on site for malaria infection using a RDT-*Pf*HRP2 supplied the Ministry of Health and a primary diagnosis was made by the health facility nurses and this was recorded on the CRF.

The performance of malaria RDT supplied by the Ministry of Health, and used in the present research, has been previously assessed in the study area (18) (23). The clinical diagnosis by the nurse was made using the International Classification of Diseases (9$^{th}$ version) (24).The participants were managed according to the Burkinabe guideline based on the WHO guideline for the integrative management of childhood illness (20).

Additional clinical specimens, i.e. blood, stool, urine and nasopharyngeal swabs, were collected for microbiology analyses and malaria microscopy (blood sample only) at the laboratory of the Clinical Research Unit of Nanoro (CRUN). Parents or guardians were asked to complete the urine and/or stool sample collection at a later stage when initial sample collection could not be completed during the enrolment procedure. When a positive culture or test justified an additional or alternative treatment, the results were communicated to the nurses in the respective health facility and if needed additional treatment was provided to the patient as soon as possible.

This study was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130).

**Laboratory procedures**

All laboratory procedures employed in this study have been described in detail previously (22). In brief, the following procedures were performed:

Malaria RDT were performed according to the manufacturer's instructions by nurses for each febrile child attending the health facilities that were used as a recruitment site. The malaria RDT used is the *Pf*HRP2 detecting SD Bioline *Pf* test (Standard Diagnostics, Hagal-Dong, Korea) supplied by the Burkinabe National Malaria Control Program (Ministry of Health). The results of malaria RDT testing were recorded as positive or negative.

Expert malaria microscopy on thin and thick blood smears was performed by two independent expert microscopists and in case of discordance (positive vs. negative, difference in *Plasmodium* species, difference in parasite density Log [difference]>Log10 or ratio>2 in case of parasite density≤400/µl and >400/µl respectively), a decisive reading was performed by a third expert.

Blood cultures were done using 1–3 ml of venous blood collected in pediatric blood culture bottles (BD BACTEC Peds Plus$^{TM}$/F, Becton Dickinson, and Company, Sparks, Maryland, USA), incubated in a BACTEC 9050 instrument (Becton Dickinson) for a total of 5 days. In case of a positive growth signal a Gram straining was done and subsequent culture was performed at 35-37°C for an additional 18-24 h.

Stool cultures were performed on Eosin-Methylene Blue (EMB) agar (for children less than 2 years of age), Hektoen agar and inoculated in selenite of sodium broth. The culture media were incubated at 35-37°C for 18-24 h, and the selenite broth at 35-37°C for 4 h before putting in culture on *Salmonella* and *Shigella* agar (*SS* agar). In addition, SD Bioline Rota/Adeno RDT (Standard Diagnostics, Inc., Korea) was used to detect Group A rotavirus or adenovirus serotype 40/41 in stool samples. Finally, fresh stool samples were used for parasitological examination for the presence of cysts, eggs, and vegetative forms of protozoa by microscopy. In case of suspicion of cysts, a second slide was prepared using lugol for confirmation.

Urine samples were tested with a dipstick (Standard Diagnostics, UroColor, Inc) for the presence of leucocytes and nitrite. If positives for these parameters, a culture of the sample was done on Cystine Lactose Electrolyte Deficient (CLED) and EMB agar and incubated at 35-37°C for 24 h.

Nasopharyngeal swabs were collected from each participant, placed in skim milk-tryptone-glucose-glycerol (STGG) broth and transported to the central CRUN laboratory in a dark storage box at room temperature. For analysis, 200 µl of this broth was vortexed and introduced in 5 ml of Todd-Hewitt (TH) broth for enrichment and incubated in two steps (before and after plating) for two times 24 h to detect *Streptococcus pneumoniae* and *Staphylococcus aureus* species (*S. pneumoniae* and *S. aureus* can be a cause of fever, but may also be carried in the nasopharynx of healthy people). Bacterial isolates from all cultures were identified by standard microbiological methods (25) and/or by Analytical Profile Index (API) biochemical test kit (bioMerieux, France).

**Data analysis**

Data were double entered using OpenClinica software. Description of qualitative and quantitative variables was performed using proportion. To assess the effect of malaria RDT on antimicrobial prescriptions, only the first antimicrobial prescription provided by the health care workers after obtaining the results of the malaria RDT was recorded. The effect of malaria RDT on the prescription of antimicrobials was assessed by cross-checking the prescription done based on malaria RDT results and malaria microscopy performed by expert microscopist. The risk ratio of a febrile child tested with malaria RDT (positive versus negative) to receive an antimicrobial prescription (i.e. antimalarial, antibiotic and antiparasitic) compared to the outcome of expert malaria microscopy was calculated using the binomial regression with a log link. The risk ratio of each antimicrobial prescribed at health facilities and referral hospital based on malaria RDT results was also calculated. Data analysis was done using STATA 13 software package® and a p-value less than 0.05 was considered statistically significant.

**Results**

*Demographic characteristics of the study population*

A total of 1099 children were included in the study. During the analysis it became apparent that information on one malaria RDT result and two malaria slide readings were missing and these were considered as missing data for the analysis. Malaria was the most frequently infection diagnosed by nurses by malaria RDT in 72.68% (798/1098) of febrile children, but only 53.69% (589/1097) of febrile children could be confirmed by expert microscopy. The second commonest cause of fever diagnosed by heath facilities nurses were respiratory tract infections (RTI) [bronchiolitis 9.2%(101/1099), pneumonia 14.47% (159/1099), other RTI 14.10% (155/1099)]. The characteristics of study population are presented in Table 1.

A small percentage of the febrile children who received antimalarial prescription got first an injectable antimalarial (artesunate or artemether) followed by artemisinin-based combination therapy (artemether-lumefantrine or artesunate-amodiaquine) for subsequent home treatment 2,11% (17/805). The majority of malaria treatment 97.88% (788/805) was artemisinin-based combination therapy as previously mentioned. For the antibiotic prescriptions, 14.58% (125/857) and 1.17% (10/857) have received respectively 2 and 3 antibiotic prescriptions, and 84.24% (722/857) received a single prescription. All antiparasitics were single prescriptions (Table 2).

**Table 1: Basic characteristic of the study population comprising of 1099 children under-5 years of age with fever (axillary temperature ≥37.5°C)**

| | No (%) |
|---|---|
| **Sex** | |
| Male | 607(55.23) |
| **Age** | |
| ≤12 months | 306(27.84) |
| **Recruitment site** | |
| Referral hospital | 294(26.75) |
| Health facilities | 805(73.25) |
| **Clinical diagnosis* (n=1099)** | |
| Malaria based on malaria RDT** | 798(72.68) |
| Septicaemia | 2(0.18) |
| Gastro-enteritis | 268(24.39) |
| Malnourished | 33(3.00) |
| Bronchiolitis | 101(9.20) |
| Pneumonia | 159(14.47) |
| Other GII | 56(5.10) |
| Other RTI | 155(14.10) |
| Urinary tract infection | 15(1.36) |
| **Laboratory findings** | |
| Malaria based on microscopy***(n=1097) | 589(53.69) |
| Bacterial bloodstream infection (n=1099) | 65(5.91) |
| Parasitic gastro-intestinal infection (n=757) | 215(28.40) |
| Bacterial gastro-intestinal infection (n=757) | 65(8.59) |
| Viral gastro-intestinal infection (n=757) | 29(3.83) |
| Urinary tract infection (n=739) | 11(1.49) |
| Common bacterial pathogens of nasopharynx (n=629) | 153(24.32) |

RDT: Rapid diagnostic test; GII: Gastro-intestinal tract infection; RTI: respiratory tract infections

* Based on RDT testing and clinical assessment by attending Health Worker

** RDT was not performed for one child N=1098).

***Two malaria slides were not performed

**Table 2: Distribution of antimicrobial prescription among febrile children**

| | Number of children who received antimicrobial prescriptions | | |
|---|---|---|---|
| **Number of antimicrobial(s) prescribed** | Antibiotics (n=857) % (n/N) | Antimalarials (n=805) % (n/N) | Antiparasitics (n=197) % (n/N) |
| **1 prescribed** | 84.25 (722/857) | 97.88 (788/805) | 100 (197/197) |
| **2 prescribed** | 14.58 (125/857) | 2.12 (17/805) | - |
| **3 prescribed** | 1.17 (10/857) | - | - |

*Influence of malaria PfHRP2-RDT on antimicrobial prescriptions*

Table 3 presents the risk of antimicrobial prescriptions according to malaria RDT results during the study period. It is evident that rural health facilities as well as the referral hospital were likely to prescribe an antimalarial in case of a positive malaria RDT, as recommended by WHO, compared to negative tested cases. Antibiotics were likely to be prescribed to negative malaria RDT. The adherence rate of the health care workers to the result of the malaria RDT-*Pf*HRP2 was 92.89% (1020/1098: 762 malaria RDT positive patients received an antimalarial treatment and 258 malaria RDT negative cases did not receive an antimalarial treatment). However, if malaria expert microscopy is considered as gold standard, the risk for febrile children tested with RDT-*Pf*HRP2 to have their initial antimalarial prescription affected (modified) was statistically significant (RR=7.74, p=0.00001). Moreover, the likelihood of antibiotic prescription in case of a negative malaria RDTs was 3 times higher compared to positive malaria RDTs and statistically significant (RR=3.57, p <0.0001).

It is apparent from Table 4 that the health care workers at the rural health facilities were more likely to prescribe antimicrobials to children who tested positive for malaria by RDT (antimalarial=96.11%; antibiotics=75.20%; antiparasitics=18.63%) than at the level of the referral hospital (antimalarial=93.37%; antibiotics=62.98%; antiparasitics=1.65%). Furthermore, the nurses in the rural health facilities were more likely to prescribe antibiotics (97.86%) and antiparasitics (26.73%) to children who tested negative by malaria RDT than attending health staff at the referral hospital (antimalarial=84.07%; antiparasitics=2.65%), except for malaria treatments (rural health facilities=7.48%; referral hospital=24.77%). As an overall trend it was found that the risk of prescribing antimalarial as well as antibiotic and antiparasitic to children with a positive malaria RDT compared to children with negative malaria RDT was higher in health facility compared to referral hospital. The risk of prescribing antimalarial in positive tested patients compared to negative malaria RDTs was 8.01 (95%CI 5.51–11.66, p=0.00001) and 6.93(95%CI=4.07–11.81, p=0.00001) for the rural health facility and referral hospital, respectively. The risk of prescribing antibiotic in case of negative malaria RDT compared to RDT positive was 11.10 (95%CI=4.18–29.43, p=0.00001) in health facility and 2.14 (95%CI=1.38–3.32, p=0.00001) in referral hospital.

**Table 3: Antimicrobial prescriptions according to malaria test results using malaria RDTs**

| Percentage of antimicrobial prescribe | Children tested positive for malaria infection by microscopy and RDT | | Children tested negative for malaria infection by microscopy and RDT | | Risk ratio of antimicrobial prescription for malaria RDT (95% CI) | p-value |
|---|---|---|---|---|---|---|
| | RDT positive, (n=798)* | Microscopy positive, (n=589)** | RDT negative, (n=300)* | Microscopy negative, (n=508)** | | |
| Antimalarial | 762(95.48) | 564(95.75) | 42(14) | 239(47.04) | 7.74(5.69 – 10.51) | <0.00001 |
| Antibiotic | 578(72.43) | 394(66.89) | 278(92.66) | 462(90.94) | 3.57(2.37 – 5.38) | <0.00001 |
| Antiparasitic | 118(14.78) | 93(15.78) | 53(17.66) | 78(15.35) | 1.16(0.90 – 1.49) | 0.240 |

*: RDT was not performed in one child.
**: Malaria microscopy was not performed in 2 children.

**Table 4: Antimicrobial prescriptions done at the of site recruitment (heath facility and referral hospital)**

| Antimicrobial | Children tested positive for malaria RDT | | Children tested negative for malaria RDT | | Risk ratio for antimicrobial prescription for health facilities patient | | Risk ratio for antimicrobial prescription for referral hospital | |
|---|---|---|---|---|---|---|---|---|
| | Health facility level, (n=617) | Referral hospital, (n=181) | Health facility level, (n=187) | Referral hospital, (n=113) | **RR (95% CI)** | **P-value** | **RR (95% CI)** | **P-value** |
| Antimalarial | 593(96.11) | 169(93.37) | 14(7.48) | 28(24.77) | 8.01(5.51–11.66) | <0.00001 | 6.93(4.07–11.81) | <0.00001 |
| Antibiotic | 464(75.20) | 114(62.98) | 183(97.86) | 95(84.07) | 11.10(4.18–29.43) | <0.00001 | 2.14(1.38–3.32) | <0.00001 |
| Antiparasitic | 115(18.63) | 3(1.65) | 50(26.73) | 3(2.65) | 1.41(1.07–1.86) | 0.0163 | 1.30(0.58–2.95) | 0.556 |

*The limitations of empiric antimicrobial prescriptions by health workers*

By cross-checking the laboratory findings (actual cause of disease) with the antimicrobials prescribed by health care workers based on the routine practice (based on the national guideline for the treatment of childhood diseases), it is evident that a large part of the febrile children who received an antibiotic prescription did actually not need such a treatment. It was that at the rural health facilities all children with a positive bacterial bloodstream infection (bBSI) (25/25) or urinary tract infection (UTI) (8/8) and 80.39% (41/51) with bacterial gastro-intestinal infection (bGII) based to laboratory results did actually receive antibiotic prescriptions. But also 93.98% of the febrile children without any infection (confirmed by laboratory testing) in the present study too actually received antibiotic prescriptions. In contrast, at the referral hospital only 75% (30/40) of children with positive bBSI, 64.28% (9/14) with bGII and 100% (3/3) with UTI did actually receive antibiotic prescription. Moreover, 86.04% (74/86) of febrile children without (laboratory confirmed) infection did also got antibiotic prescriptions (Table 5).

**Table 5: Antibiotic and antiparasitic prescriptions at the referral hospital and health facilities according to laboratory findings.**

| | bBSI (n=65) n/N (%) | bGII (n=65) n/N (%) | pGII (n=215) n/N (%) | vGII (n=29) n/N (%) | UTI (n=11) n/N (%) | CBPN (n=153) n/N (%) | No infection* (n=269) n/N (%) |
|---|---|---|---|---|---|---|---|
| **Referral hospital** | 40/65(61.54%) | 14/65(21.54) | 40/215(18.60) | 6/29(20.69) | 3/11(27.27) | 41/153(26.80) | 86/269(31.97) |
| **Antimalarial** | 31/40(77.5) | 8/14(57.14) | 29/40(72.5) | 3/6(50) | 3/3(100) | 21/41(51.21) | 30/86(34.88) |
| Antibiotic | 30/40(75) | 9/14(64.28) | 29/40(72.5) | 6/6(100) | 3/3(100) | 33/41(80.48) | 74/86(86.04) |
| Antiparasitic | 0/40(0) | 0/14(0) | 1/40(2.50) | 1/6(16.67) | 0/3(0) | 1/41(2.43) | 2/86(2.32) |
| | | | | | | | |
| **Health facilities** | 25/65(38.46) | 51/56(78.46) | 175/215(81.40) | 23/29(79.31) | 8/11(72.73) | 112/153(73.20) | 183/269(68.02) |
| **Antimalarial** | 16/25(64) | 39/51(76.47) | 129/175(73.71) | 13/23(56.52) | 7/8(87.5) | 85/112(75.89) | 86/183(46.99) |
| Antibiotic | 25/25(100) | 41/51(80.39) | 141/175(80.57) | 20/23(86.95) | 8/8(100) | 97/112(86.60) | 172/183(93.98) |
| Antiparasitic | 5/25(20.00) | 16/51(31.37) | 30/175(17.14) | 4/23(17.39) | 1/8(12.5) | 20/112(17.85) | 47/183(25.68) |

bBSI: bacterial bloodstream infection; bGII: bacterial gastro-intestinal infection; vGII: viral gastro-intestinal infection; UTI: urinary tract infection; CBPN: common bacteria pathogens of nasopharynx.

*Including malaria infection

**Discussion**

A rapid diagnostic test based on the detection of *P. falciparum* specific HRP-2 antigen is one of the few diagnostic tools available for health care workers in many resource limited settings in malaria endemic settings to assist them in differentiating between malaria fever and other causes of fever in many malaria. As previously reported, HRP-2 based RDTs displayed a low specificity (59%) in the current study area (18). This low specificity of HRP2 based RDT was also reported in other malaria endemic areas (13) (26) (27) (28). Therefore, expert microscopy was performed in the present study in the central laboratory of CRUN to further determine (by cross-checking) whether antimicrobials were appropriately prescribed by the attending health care staff. Despite the good attitude of health care workers to nearly always use malaria RDT in case of a febrile patient and act accordingly to the result of diagnostic testing (92.89 of adherence of health care workers to malaria RDT), the prescription of antimalarials is affected by the performance of the malaria RDT used in the study area (23). Due to the persistence of HRP2 antigen after successful antimalarial treatment, malaria infection is overestimated (as a result of a false positive RDT) leading to an inappropriate prescription of antimalarials (13) (14) (15) (16). Furthermore, even if the diagnosis malaria was established by RDT, there was a tendency to prescribe antibiotics next to antimalarials too. Moreover, and even worse, the prescription of antibiotics becomes almost systematic when a malaria infection could be excluded. The majority of outpatients were visiting the rural health facilities where there is a lack of laboratory facilities that can confirm the actual cause of infection and this explains the inappropriate prescription of antibiotics at this level. In contrast, at the level of the referral hospital these facilities are available, but there the attending hospital health care workers might not feel confident to postpone antibiotic treatments in febrile children until the microbiology results become available in fear of treating a potentially life threating, but treatable, bacterial infection too late or overlooking such an infection. Given the fact that health care workers tend to adhere well to malaria RDT results as reported in our study area [this study and previous study by Ruizendaal E. et *al.* (29)], it is most probable that health system may be able to deal with the problem of inappropriate prescription of antibiotics as long as the health care workers get the appropriate diagnostic tools. Therefore, the development of (near) point-of-care tests to screen for other causes of fever becomes essential to guide appropriate antibiotic prescriptions.

The inappropriate prescription of antibiotics and antimalarial treatments observed in the present study could be a consequence of the rightful fear of health care workers to miss a treatable bacterial infection. Indeed, a significant number of potentially treatable etiologies

were missed in the present study and others (18) (30). Despite the tendency of systematic prescription of antibiotics to febrile children, an important number of children with bacterial infections did not receive appropriate treatment mainly at the referral hospital at the first contact compared to the rural health facilities. This observation can be explained by the availability at referral hospital of equipped laboratory allowing for a better diagnostic compared to health facilities where malaria RDT is the only diagnostic tool available. Moreover, in Burkina Faso, nurses working at health facilities are responsible for the primary management of patients, while at the referral hospital there are medical doctors who are better trained to make a specific prescription. The fear to overlook a potential bacterial infection by sending a sick child back home without prescribing medication leads to the antibiotic prescriptions to an important part of febrile children without bacterial infections. This tendency of systematic prescription of antibiotics to febrile children is considered in many setting to be more a social and behavioral issue than a medical problem (31). To tackle this problem, there is a need to evaluate and understand the context in which the (over) prescriptions occur. Moreover, more diagnostic tools should become available that can be implemented in resource limited settings that can aid proper antibiotics prescriptions and thereby reducing the risk of inappropriate prescription and of emerging of antibiotic resistance.

Antiparasitics drugs were not much prescribed in this study. Based on the laboratory findings, there was a high under-prescription of antiparasitics as actually a significant number of febrile children who actually had gastrointestinal parasites did not receive appropriate treatment. Most likely, the more obscure symptoms associated with a parasitic gastro-intestinal infection, which in most cases do not cause fever, are probably overlooked in favor of other febrile etiologies that are more readily diagnosed.

Despite the recommendation of the WHO to treat all children with a positive malaria RDT or microscopy result, few cases (4.52%) of febrile children who had a malaria positive RDT did not receive antimalarials. The reason for this non adherence was not assessed in the present study. Possibly, complementary information on previous drug use provided by the parents/guardians to the attending nurses suggested that these children did not require antimalarial prescriptions and that the RDT were positive in these cases was interpreted as a consequence of persisting HRP2 antigen (up to 4 weeks or even more) after successful treatment for malaria (13) (14) (15) (16). In contrast, 14% of the children with a malaria RDT negative result still received antimalarial prescriptions. It has not been determined in the present study what the rationale was behind this non-compliance. This lack of adherence to the

protocols in place for the management of malaria has also been reported elsewhere (32). Therefore, it is of utmost importance to further study the motivation of health care workers to not adhere to this protocol recommended by the WHO and the National Malaria Control Program (NMCP) and this should preferably be done, in combination with to further training and education of the health practitioners.

A possible limitation of our study could be the lack of confirmation of pneumonia cases presumptively diagnosed by health care workers. Although the nasopharynx of some children was colonized by common bacterial pathogens, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, a relation of their presence with ongoing fever has not been established (22). Therefore, it might be possible that some of these children did not have pneumonia. Moreover, RTI are the second cause of fever diagnosed presumptively by health facility nurses after malaria in the present study. The WHO guideline recommends the prescription of antibiotics to children with (suspected) pneumonia but not to children with acute bronchitis (1) (20) (33) (34). In the present study, a radiological confirmation of pneumonia could not be done in children suspected of RTI, because this diagnostic technology was not available at the participating health centres. Another possible limitation of the present research is the potential under-diagnosis of the number of UTI and GII cases as some urine and stool samples were not collected during recruitment. Finally, viral etiologies are known to be responsible for a significant number of fever episodes in children (17) (35) (36), but these were not studied in the present study as the CRUN laboratory does not have facilities to perform virus identification. Although viral febrile infections do not need antibiotic treatment, they can also be a cause of over prescriptions as there is fear of overlooking potential bacterial infections. A rapid diagnostic test (or combination of tests) that can broadly differentiate between bacterial and viral infections would be very helpful in this respect (37) (38) (39).

**Conclusion**

Despite the correct attitude of health care workers to treat malaria according to a diagnostic test result, antimalarials and antibiotics are still inappropriately prescribed by nurses. The low specificity of the malaria RDT used and the absence of practical tools to diagnose bacterial infections could be important causes of this inadequate prescription practice. In addition, the fear to delay antibiotic treatment or to overlook a treatable bacterial infection, leads to the inappropriate prescription of antibiotics. The health system is likely to deal with the problem of inappropriate prescription if appropriate diagnostic tools are developed and implemented.

**List of abbreviations**

API: Analytical Profile Index

bBSI: bacterial bloodstream infection

CMA: Centre Medical avec Antene Chirugicale

bGII: bacterial gastro-intestinal infection

CI: Confidence Interval

CLED: Cystine Lactose Electrolyte Deficient

CRF: Case Report Form

CRUN: Clinical Research Unit of Nanoro

EMB: Eosin-Methylene Blue

FIND: Foundation for Initiative New Diagnostics

HRP-2: Histidine-Rich Protein-2

NMCP: National Malaria Control Program

*Pf*: *Plasmodium Falciparum*

RDT: Rapid Diagnostic Test

RR: Risk Ratio

RTI: respiratory tract infections

S. aureus: *Staphylococcus aureus*

S. pneumoniae: *Streptococcus pneumoniae*

SD: Standard Diagnostic

*SS*: *Salmonella* and *Shigella*

STGG: Skim Milk-Tryptone-Glucose-Glycerol

TH: Todd-Hewitt

UTI: Urinary Tract Infection

WHO: World Health Organization

**Declarations**

*Ethics approval and consent to participate*

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent for the participation of the children was obtained from parents or legal guardians prior to enrolment in the study.

*Consent for publication*

Not applicable.

*Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors declare that they have no competing interests

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*Authors' contributions*

MB, FK, MT HS, PM, HT and MBvH conceived and designed the study. FK, MT, AS, PL and MB supervised patient inclusion, signature of informed consent and diagnostic specimen collection by study nurses. MB, KF, MT and PL performed/supervised the laboratory analyses. MB and FK analyzed the data under the supervision of a biostatistician. MB, FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript.

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# *Chapter 7*

## *Can clinical signs or symptoms combined with basic hematology data be used to predict the presence of bacterial infections in febrile children under 5 years?*

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**Abstract:**

**Background:** Infectious diseases in children living in resource-limited settings are often presumptively managed on the basis of clinical signs and symptoms. Malaria is an exception. However, the interpretation of clinical signs and symptoms in relation to bacterial infections is often challenging, which may lead to an over prescription of antibiotics when a malaria infection is excluded. The present study aims to determine the association between clinical signs and symptoms and basic hematology data, with laboratory confirmed bacterial infections.

**Methods:** A health survey was done by study nurses to collect clinical signs/symptoms in febrile (axillary temperature ≥37.5°C) children under-5 years of age. In addition, blood, stool and urine specimen were systematically collected from each child to perform bacterial culture and full blood cell counts. To determine the association between a bacterial infection with clinical signs/symptoms, and if possible supported by basic hematology data (hemoglobin and leucocyte rates), a univariate analysis was done. This was followed by a multivariate analysis only on those variables with a p-value $p<0.1$ in the univariate analysis. Only a p-value of $< 0.05$ was considered as significant for multivariate analysis.

**Results:** In total, 1099 febrile children were included. Bacteria were isolated from clinical specimens (blood-, stool- and urine- culture) of 127 (11.6%) febrile children. Multivariate logistical regression analysis revealed that a general bacterial infection (irrespective of the site of infection) was significantly associated with the following clinical signs/symptoms: diarrhea (p=0.003), edema (p=0.010) and convulsion (p=0.021). Bacterial bloodstream infection was significantly associated with fever>39.5ºC (p=0.002), diarrhea (p=0.019) and edema (p=0.017). There was no association found between bacterial infections and basic haematological findings. If diarrhea and edema were absent, a good negative predictive value (100%) of a bacterial bloodstream infection was found, but the positive predictive value was low (33.3%) and the confidence interval was very large (2.5–100; 7.5–70.1).

**Conclusion:** Our study demonstrates that clinical signs and symptoms, combined with basic hematology data only, cannot predict bacterial infections in febrile children under-5 years of age. The development of practical and easy deployable diagnostic tools to diagnose bacterial infections remains a priority.

**Key words:** fever, children, bacteria, malaria, signs, symptoms.

**Background**

In resource-limited settings, infectious diseases are mainly presumptively managed on the basis of clinical signs and symptoms (1) (2). However, the interpretation of these clinical signs and symptoms to make a diagnosis of bacterial infections is often challenging, and leads to an overuse of antibiotics in fear of overlooking bacterial infections. This practice strongly contributes to the development of drug resistance (3). This is perhaps not the case for malaria for which rapid diagnostic tests (RDTs) are available (4). This approach has been successful to control malaria in endemic areas (5).

However, the use of malaria RDT leaves a significant part of the febrile, non-malaria, patient population undiagnosed. Clinicians that work in areas without laboratory facilities can only manage their patients on the basis of clinical signs and symptoms, which can sometimes be supported by simple tests for hemoglobin and white blood cell count (4) (6) (7) (8). Therefore, a proper assessment of the value of clinical signs and symptoms to predict bacterial infections could have a major practical impact. Previous studies tried to define infections according to the localization of the infection, for example chest or intestines (9) (10) (11) (12) (13). However, these definitions were not focused on the infecting pathogens (bacterial, viral or parasitic). This has left a gap in fever management and explains increased numbers of antibiotic prescriptions, which is nowadays replacing the inappropriate use of anti-malarials (5). Moreover, the interpretation of clinical signs and symptoms could vary between areas as the epidemiology of infectious diseases are different (14) (15). For the management of febrile children, it could therefore be helpful to assess the relationship between bacterial infections and clinical signs and symptoms (supported with some simple basic hematology data).

**Methods**

**Study site**

The study was performed in the health district of Nanoro, located in the Center-West region of Burkina Faso at about 100 km from Ouagadougou, the capital city. The data were collected in four peripheral health facilities (Nanoro, Godo, Nazoanga and Seguedin) and at the Pediatric Department of the district referral hospital, the Centre Médical avec Antenne Chirurgicale (CMA) Saint Camille of Nanoro. The peripheral health facilities are the first medical point of contact within the community for the management of less complicated medical cases. In this setting primary health care is provided by nurses and only severe cases are transferred to the referral hospital. The referral hospital is managing the more complex cases and a pediatrician

is available. Clinical signs and symptoms, and medical history, are the only information available to the attending health workers in these health facilities to make a primary diagnosis and to install disease management, except for malaria for which a rapid diagnostic test is available. Some basic laboratory data (hematology) can be made available in the referral hospital, but there is no possibility to perform for example blood cultures. Malaria is the first cause of consultation in children under-5 years of age and occurs mainly during the rainy season which runs from July to November (16). Vaccination against *Haemophilus influenzae* type b was introduced into the extended program of immunization (EPI) in Burkina Faso in January 2006 (16). This program was extended with the introduction of vaccination against pneumococcal disease and rotavirus in October 2013 (Source: Ministry of Health, Burkina Faso).

**Study procedure**

A cross-sectional study was conducted between January-December 2015 and April-October 2016. All children attending the pediatric service of district referral hospital or one of peripheral health facilities were routinely screened, but only children with a documented age under-5 years and axillary temperature ≥37.5°C were invited to participate in the present study. Written informed consent was obtained from accompanying parent or legal guardian. Standard Case Report Forms (CRF) were used to record clinical signs and symptoms based on clinical examination and history, together with some basic demographic information. Nurses, trained by a pediatrician and with experience in working in clinical research, performed the primary assessment of the study cases and collected the clinical signs and symptoms. The following symptoms were systematically collected by the nurses: cough, diarrhea and vomiting. The following signs were also systematically assessed during the physical examination of febrile children: edema, dehydration, jaundice, pallor of conjunctiva, bronchial crepitation's, splenomegaly and hepatomegaly. Primary diagnosis was done according to the International Classification of Diseases ($9^{th}$ version) (17).

Next to the standard clinical examination, blood, stool and urine samples were systematically collected as describe previously, for cultures and full blood cell count (18). The clinical specimens were analyzed at the microbiology laboratory of the Clinical Research Unit of Nanoro (CRUN). The microbiology laboratory of CRUN is subjected to internal quality control (according to a standard auditing protocol). Furthermore, it is also subjected to external and international quality control audits organized by the National Institute for Communicable Diseases (NICD). Bacterial bloodstream infection (BSI) or bacteremia, bacterial gastro-

intestinal infection (GII) or bacterial gastroenteritis and urinary tract infection (UTI) or bacteriuria were the bacterial infections considered in this study. Children with positive bacterial culture (BSI, GII or UTI) were regrouped in general bacterial infections to assess the association between clinical signs and symptoms supported by basic hematology data with bacterial infection (all).

Patients were managed firstly according to the Burkinabe national guidelines based on WHO guidelines for the integrated management of childhood illness (19). However, when available, the additional laboratory data were communicated to the appropriate health facilities or the district hospital as soon as these results became accessible and if needed the patient management was changed free of charge. The complementary diagnostic information and treatment provided were not used in the analysis. However, it is important to note that the outcome of diseases was not collected after the inclusion.

The study protocol was approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130).

**Laboratory investigations**

*Microbiological culture*

Around 1–3 ml of venous blood was collected into pediatric culture bottle (BD BACTEC Peds Plus$^{TM}$/F, Becton Dickinson and Company, Sparks, Maryland, USA) and subsequently incubated in an automated culture system, a BACTEC 9050 instrument (Becton Dickinson), for a total of 5 days. One culture bottle was used per participant. Positive cultures were next Gram stained and further cultured on standard media like Eosin-Methylene Blue (EMB) agar, 5% Sheep Blood (SB) agar (bioMérieux, Marcy-l'Etoile, France) and chocolate gelose (CG) + isoVitalex (CG + IVX) agar, and incubated at 35–37°C for 24 hours under atmospheric conditions for EMB and at dioxide of carbon ($CO_2$) for SB agar and chocolate + isoVitalex agar. Standard microbiology methods like those described in Mackie and McCartney Practical Medical Microbiology (20) and Analytical Profile Index (API) biochemical test kit (bioMerieux, France) were used to identify suspected pathogens. Contaminated blood cultures were reported as "negative blood culture".

Fresh stool samples were screened for enterobacteriaceae-pathogens, by plating on EMB agar (only done for children under-24 months of age to check for enteropathogenic *Escherichia coli*), Hektoen agar, and sodium selenite broth, and incubated at 35–37°C. The sodium selenite

broth was sub-cultured on *Salmonella* and *Shigella* agar (SS agar) after 4 hours of incubation. Suspected pathogens were identified as described above.

Collected urine was first tested with a dipstick (Standard Diagnostics, UroColor, Inc,, Korea) and if positive for leucocytes and nitrite, the sample was plated on CLED (Cysteine Lactose Electrolyte Deficient) and EMB agar and incubated at 35-37°C during 24 hours. Only pure bacterial growth (i.e. only one species grown on the plate) of more than $10^5$ colonies forming units (CFU)/ml was regarded as significant bacteriuria. Bacteria count $\leq 10^5$ was regarded as negative and mixed growths (growth of more than one species in a sample) was regarded as contaminated and therefore disregarded. In that case, a new urine sample was not collected and considered as a missing sample. Suspected pathogens were identified with standard microbiology methods as described above.

Venous blood was collected in in ethylene-diamine tetra-acetic acid (EDTA) tubes. The full blood cell counts were assessed by using Sysmex XS1000i (Sysmex Corporation, Kobe, Japan) according to manufacturer's instructions.

**Data analysis**

Double data entry using OpenClinica software was done. Data analysis was done using R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). The mean and median were used for continuous variables. For the categorical data and dichotomous variables, stratified by clinical signs and symptoms and basic laboratory data, percentage was used. In order not to miss any potential associations between clinical signs and symptoms, and basic hematology data (hemoglobin and leucocyte rates), we included all the clinical signs and symptoms, and basic hematology data in the data analysis. For the factor depending of age including weight, height and mid arm circumference, the Z-score was calculated for each child. Children with a 2 × SD for weight, height and perimeter brachial were considered as moderate malnourished and over 3 × SD like severe malnourished.

To assess whether clinical signs and symptoms can diagnose bacterial infections investigated in the present study in febrile children under-5 years of age, the following analysis was done. Firstly, univariate logistic regression analyses were preformed to identify the subset of independent variables that were linked to general bacterial infections, as well as bacterial BSI, bacterial GII and UTI separately. In order not to miss any relevant clinical characteristics obtained at physical examination, we used all the clinical signs and symptoms reported by nurses as well as basic hematology data performed at hematology-biochemical laboratory

CRUN for the univariate analysis. Only the variables with a significant level of p≤0.1 were considered to be candidate variables for multivariate logistic regression analysis. For the determination of the association in multivariate analysis, general bacterial infections as well as bacterial BSI, bacterial GII, and UTI were adjusted for potential confounding factors (age, sex and weigh). The variables significantly associated to general bacterial infection in the univariate analyses were subsequently included in the multivariate analysis for general bacterial infections as well as bacterial BSI, bacterial GII and UTI. We calculated the association of clinical signs and symptoms, and basic hematology data with bacterial infections for the multivariate logistical regression by estimated the p-value (p≤0.05). The positive and negative predictive value of the combination of the variables with significant level after the multivariate analysis were evaluated by testing these combinations in study population.

**Results**

In total, 1099 febrile children under-5 years of age were included in the study. Males represented 55.2% (607/1099) of the study population. The median age was 21 months (Interquartile [IQR]: 12–32) and 27.8% (306/1099) were children under-12 months. The main clinical signs and symptoms reported were cough and diarrhea in 43.5% (478/1099) and 37.8% (371/1099) of the cases, respectively (Table 1). According to the z - score calculations no cases of severe malnutrition (z-score>3SD) were found (Table 1).

The prevalence of the bacterial infections investigated in the present study is reported in table 1. After laboratory analyses, 1% (11/1099) of children had at least two infections at the same time (8 had BSI and GII; 2 had BSI, GII and UTI; 1 had GII and UIT). All these infections were taken into consideration whilst doing the data analysis. For bacterial BSI, *Salmonella ssp* were the most frequently isolated pathogens (78.5%; 51/65), followed by *Streptococcus pneumoniae* and *E. coli* in 6.2% (4/65) in both cases, *Staphylococcus aureus* and *Neisseria meningitides* in 3.1% (2/65) in both cases, *Haemophilus influenzae* B and *Enterobacter agglomerans* in 1.5% (1/65). For bacterial GII, enteropathogenic *E. coli* was isolated in 50.8% (33/65), *Salmonella spp* in 44.6% (29/65) and *Shigella* in 4.6% (3/65). *E. coli* was the only species isolated from UTI. Three pediatric bottle flagged positive for growth the cultures were due to contamination.

Criteria for hospital admission were not defined in the present study. The referrals and admissions were done according to the routine practice (mainly based on severity of clinical

symptoms and suspected disease) In the present study, 17.9% (197/1099) of the recruited febrile children were hospitalized by health professionals. A bacterial infection was found in 3.5% (38/1099) of these children and 14.5% (159/1099) was negative for a bacterial infection. The admission rate was almost two-time higher for children with a confirmed bacterial infection 29.9% (38/127) compared to those that were negative for a bacterial infection 16.4% (159/972) (Table 1).

**Table 1: Clinical and basic laboratory data characteristics of study population, bacterial infected (all) and uninfected group, and bacterial infected group by infection (BSI, GII and UTI)**

| Characteristic | Study population | Positive and negative bacterial infection | | Positive bacterial infections | | |
|---|---|---|---|---|---|---|
| | N=1099 | Positive bacterial infections | Negative bacterial infections | BSI | GII | UTI |
| **Demographic data** | | | | | | |
| **Total, n (%)** | 1099(100.0) | 127(11.6) | 972(88.4) | 65(5.9) | 65(8.60) | 11(1.5) |
| **Male, n (%)** | 607(55.2) | 59(46.5) | 548(56.4) | 34(52.3) | 28(43.1) | 5(45.5) |
| **Age in months, median (IQR)** | 21.0 (12.0-32.0) | 19.0 (12.0-25.0) | 21.0 (12.0-33.0) | 21.0 (13.0-30.0) | 17.0 (13.0-23.0) | 13.0 (7.0-21.5) |
| **Age** ≤12 months (%) | 306(27.8) | 33(26.0) | 273(28.1) | 16(24.6) | 16(24.6) | 5(45.5) |
| **Age >12 months (%)** | 793(72.2) | 94(74.0) | 699(71.9) | 49(75.4) | 49(75.4) | 6(54.5) |
| **Z-score Weight/age<2SD in kg/month, mean (SD)** | 9.7(3.0) | 8.9(2.4) | 9.8 (3.1) | 9.1(2.8) | 8.9(1.9) | 7.70(1.3) |
| **Z-score Height/age<2SD in cm/month, mean (SD)** | 81.0(12.3) | 79.1(10.1) | 82.3(12.6) | 80.4(11.1) | 77.4(11.7) | 68.2(19.6) |
| **Z-score MUAC in mm/age, mean (SD)** | 125.1(57.8) | 128.9(82.2) | 124.6(53.9) | 132.3(109.1) | 123.6(39.7) | 120.0(35.8) |
| **Admitted to referral hospital** | 197(17.9) | 38(29.9) | 159(16.4) | 29(44.61) | 11(16.9) | 3(27.3) |
| **Vitals** | | | | | | |
| **Temperature in,** °C, mean (SD) | 38.7(0.8) | 38.8(0.8) | 38.7(0.8) | 38.9(0.80) | 38.7(0.80) | 38.5(0.87) |
| **[37.5**°C-38.5°C], % | 541(49.2) | 54(42.5) | 487(50.1) | 26(40.0) | 28(43.1) | 5(45.5) |
| **]38.5**°-39.5°C], % | 394(35.9) | 45(35.4) | 349(35.9) | 22(33.8) | 26(40.0) | 5(45.5) |
| **>39.5**°C (%) | 164(14.9) | 28(22.0) | 136(14.0) | 17(26.2) | 11(16.9) | 1(9.1) |
| **Fever (**≥38.5°C), % | 558(50.8) | 73(57.5) | 485(49.9) | 39(60.0) | 37(56.9) | 6(54.5) |
| **Respiratory rate /min, mean (SD)** | 38.1 (15.8) | 36.7(4.9) | 38.3 (16.7) | 37.02 (4.4) | 36.22 (5,1) | 38.82 (3.6) |
| **Laboratory data** | | | | | | |
| **Hemoglobin rate, n (%)** | | | | | | |
| **<8g/dl** | 300(27.3) | 52(40.9) | 248(25.7) | 39(60.0) | 18(27.7) | 5(45.5) |
| ≥8g/dl-<11g/dl | 630(57.3) | 61(48.0) | 569(58.9) | 19(29.2) | 39(60.0) | 4(36.4) |
| **≥11g/dl** | 163(14.8) | 14 (11.0) | 149(15.4) | 7(10.8) | 8(12.3) | 2(18.2) |
| **No data** | 6(0.5) | - | - | - | - | - |
| **White blood cells, n (%)** | | | | | | |
| **<4.10$^3$ cells/mm$^3$** | 26(2.4) | 5(3.9) | 21(2.2) | 3(4.6) | 2(3.1) | 0.0 |
| **≥**4.10$^3$ **- < 12.10$^3$ cells/mm$^3$** | 587(53.4) | 72(56.7) | 515(53.4) | 32(49.2) | 41(63.1) | 36.4 |
| **≥12.10$^3$ cells/mm$^3$** | 478(43.5) | 50(39.4) | 428(44.4) | 30(46.2) | 22(33.8) | 63.6 |
| **No data** | 8(0.7) | - | - | - | - | - |
| **Signs and symptoms, n (%)** | | | | | | |
| **Cough** | 478(43.5) | 56(44.1) | 422(43.4) | 33(50.8) | 26(40.0) | 3(27.3) |
| **Diarrhea** | 371(37.8) | 57(44.9) | 314(32.3) | 29(44.6) | 27(41.5) | 8(72.7) |
| **Vomiting** | 201(18.3) | 25(19.7) | 176(18.1) | 11(16.9) | 16(24.6) | 2(18.2) |
| **Jaundice** | 10(0.9) | 0(0.0) | 10(1.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| **Edema** | 10(0.9) | 5(3.9) | 5(0.5) | 4(6.2) | 1(1.5) | 0(0.0) |
| **Pallor of conjunctiva** | 102(9.3) | 21(16.5) | 81(8.3) | 18(27.7) | 4(6.2) | 2(18.2) |
| **Dehydration** | 37(3.4) | 8(6.3) | 29(3.0) | 6(9.2) | 1(1.5) | 1(9.1) |
| **Convulsion** | 20(1.8) | 6(4.7) | 14(1.4) | 4(6.2) | 2(3.1) | 0(0.0) |

BSI: Bloodstream infection; GII: Gastro-intestinal infection; UTI: Urinary tract infection; min: minute; mm: millimeter; MUAC: Mid-Upper Arm Circumference

Table 2 shows the association of a general bacterial infection, bacterial BSI, UTI or bacterial GII, with the clinical signs and symptoms, and basic hematology data in univariate analysis. The clinical signs and symptoms significantly associated in the univariate analysis to bacterial infections were high axillary temperature (≥39.5ºC), diarrhea, dehydration, edema, convulsion, pallor conjunctiva, splenomegaly and hepatomegaly. For the basic hematology data only hemoglobin<8g/dl was significantly associated in the univariate analysis to bacterial infections ($p<0.1$). Children with moderate malnutrition according to z-score calculation (weight/age and height/age) were also prone to have a general bacterial infection. Gender and age were also associated with a general bacterial infection ($p<0.1$). Based on the information obtained from the bacterial cultures, it was found that bacterial BSI was significantly associated in the univariate analysis to diarrhea, dehydration, edema, convulsion, pallor conjunctiva, splenomegaly, hepatomegaly and hemoglobin<8g/dl ($p<0.1$). Age and moderate malnutrition according to z-score calculation (weight/age and height/age) were significantly associated to UTI, after the univariate analysis. Based on stool culture, bacterial GII was associated to gender, age and moderate malnutrition according to z-score calculation (weight/age and height/age) in univariate analysis ($p<0.1$) (Table 2).

**Table 2: Clinical signs and symptoms and basic laboratory results associated to bacterial infection in univariate analysis**

| | Bacterial infected group | | BSI | | UTI | | GII | |
|---|---|---|---|---|---|---|---|---|
| **Clinical signs and symptoms** | OR (IC 95%) | P value | OR (IC 95%) | P value | OR (IC 95%) | P value | OR (IC 95%) | P value |
| Male | 1.04(1.00;1.08) | **0.034*** | 1.13(6.69;1.86) | 0.629 | 1.43(0.46;4.40) | 0.537 | 1.66(1.01;2.74) | **0.046*** |
| Age, month, median (IQR) | 1.00(1.00;1.00) | **0.050*** | 1.00(0.98;1.02) | 0.833 | 0.95(0.90;1.00) | **0.069*** | 0.98(0.96;1.0) | **0.026*** |
| >12 months (%) | 1.01(0.96;1.05) | 0.619 | 1.19(0.67;2.10) | 0.555 | 0.50(0.16;1.55) | 0.227 | 1.19(0.67;2.10) | 0.555 |
| Z-score Weight/age<2SD in kg/month, kg/age, mean (SD) | 0.99(0.98;1.00) | **0.002*** | 0.93(0.85;1.02) | 0.123 | 0.75(0.58;0.96) | **0.023*** | 0.90(0.82;0.98) | **0.021*** |
| Z-score Height/age<2SD in cm/month, cm/kg, mean (SD) | 1.00(1.00;1.00) | **0.068*** | 1.00(0.96;1.02) | 0.683 | 0.95(0.90;1.00) | **0.049*** | 0.98(0.96;1.00) | **0.072*** |
| Z-score MUAC<2SD in mm/age mm, mean (SD) | 1.00(1.00;1.00) | 0.430 | 1.00(1.00;1.01) | 0.308 | 1.00(0.99;1.01) | 0.758 | 1.00(0.99;1.00) | 0.821 |
| Cough | 1.00(0.97;1.04) | 0.884 | 1.36(0.83;2.23) | 0.229 | 0.53(0.16;1.80) | 0.308 | 0.86(0.52;1.43) | 0.563 |
| Diarrhea | 1.06(1.02;1.10) | **0.004*** | 1.61(0.98;2.66) | **0.061*** | 4.53(1.33;15.42) | **0.016*** | 1.41(0.85;2.34) | 0.178 |
| Dehydration | 1.11(1.00;1.23) | **0.051*** | 3.08(1.25;7.61) | **0.015*** | 2.56(0.31;16.38) | 0.422 | 0.52(0.10;2.71) | 0.434 |
| Vomiting | 1.01(0.96;1.06) | 0.666 | 0.91(0.47;0.75) | 0.773 | 1.00(0.24;4.08) | 0.993 | 1.48(0.83;2.65) | 0.183 |
| Edema | 1.47(1.21;1.80) | **<0.001*** | 9.48(2.67;33.67) | **0.001*** | 0.70(0.02;30.91) | 0.854 | 1.55(0.23;10.33) | 0.653 |
| Jaundice | 0.89(0.72;1.09) | 0.251 | 0.35(0.02;7.30) | 0.497 | 0.70(0.02;30.91) | 0.854 | 0.35(0.02;7.30) | 0.497 |
| Convulsion | 1.21(1.05;1.39) | **0.009*** | 3.74(1.23;11.40) | **0.020*** | 0.57(0.02;18.13) | 0.752 | 1.65(0.40;6.77) | 0.489 |
| Pallor of conjunctiva | 1.10(1.03;1.18) | **0.003*** | 4.20(2.34;7.52) | **<0.001*** | 1,94(0.45;8.47) | 0.376 | 0.65(0.24;1.73) | 0.387 |
| Axillary temperature [38.5,39.5] | 1.01(0.97;1.05) | 0.496 | 1.16(0.65;2.05) | 0.496 | 1.35(0.42;4.34) | 0.505 | 1.28(0.75;2.20) | 0.900 |
| Axillary temperature [39.5,42] | 1.07(1.02;1.14) | **0.013*** | 2.23(1.19;4.18) | 0.496 | 0.72(0.12;4.36) | 0.505 | 1.30(0.64;2.63) | 0.900 |
| Fever (≥38.5°C), | 0.99(0.93;1.01) | 0.108 | 0.68(0.41;1.12) | 0.131 | 0.87(0.28;2.69) | 0.812 | 0.77(0.47;1.28) | 0.313 |
| Bronchial congestion | 0.97(0.85;1.10) | 0.602 | 1.15(0.37;3.62) | 0.807 | 1.96(0.28;13.72) | 0.499 | 0.78(0.21;2.92) | 0.712 |
| Splenomegaly | 1.18(1.07;1.29) | **<0.001*** | 6.80(3.40;13.57) | **0.000*** | 0.39(0.02;8.71) | 0.556 | 0.718(0.19;2.66) | 0.620 |
| Hepatomegaly | 1.10(1.01;1.19) | **0.030*** | 3.50(1.69;7.23) | **0.001*** | 0.35(0.02;7.28) | 0.500 | 0.59(0.16;2.15) | 0.423 |
| **Basic Laboratory data**†† | | | | | | | | |
| Hemoglobin <8 | 1.09(1.03;1.16) | **0.004*** | 3.21(1.46;7.07) | **0.004*** | 1.38(0.32;5.98) | 0.663 | 1.21(0.54;2.74) | 0.645 |
| Hemoglobin [8-11] | 1.01(0.95;1.07) | 0.696 | 0.68(0.30;1.57) | 0.366 | 0.56(0.13;2.4) | 0.444 | 1.25(0.60;2.65) | 0.552 |
| Leucocytes <4 | 1.07(0.95;1.22) | 0.279 | 2.07(0.61;7.01) | 0.241 | 0.59(0.018;19.90) | 0.771 | 1.10(0.28;4.33) | 0.90 |
| Leucocytes≥12 | 0.98 (0.94;1.02) | 0.361 | 1.15(0.69;1.91) | 0.590 | 2.04(0.64;6.52) | 0.227 | 0.65(0.38;1.10) | 0.106 |

*: Statistically significant

†: **According to nurse's appreciations**

††: hemoglobin value was g/dl; leucocyte value was $10^3$/µl

BSI: Bloodstream infection; GII: Gastro-intestinal infection; UTI: Urinary tract infection.

**Note:** The different data were adjusted for gender, age and weigh during the multivariate analysis.

MUAC: Mid-Upper Arm Circumference

The multivariate logistical regression analysis revealed that a general bacterial infection was significant associated to the following clinical signs and symptoms: high axillary temperature ≥39.5ºC (p=0.002), diarrhea (p=0.003), edema (p=0.010) and convulsion (p=0.021) (See table 3). Based on infection type, bacterial BSI was significantly associated with high axillary temperature ≥39.5ºC [p=0.002; IC 95%=(1.51;5.97)], diarrhea [p=0.019; IC 95%=(1.12;3.46)] and edema [p=0.017; IC 95%=(1.38;26.39)]. Bacterial GII was not associated with clinical signs and symptoms, and basic laboratory data according to this second criteria. The multivariate analysis was adjusted for age, gender and weight, which are confounding factors.

**Table 3: Clinical signs and symptoms and basic laboratory results associated to bacterial infection in multivariate logistic regression analysis**

| | Bacterial infected group | | BSI | | UTI | | GII | |
|---|---|---|---|---|---|---|---|---|
| **Clinical signs and symptoms** | OR (IC 95%) | P value | OR (IC 95%) | P value | OR (IC 95%) | P value | OR (IC 95%) | P value |
| Z-score Weight/age<2SD in kg/month, kg/age, mean (SD) | 0.95(0.68 ;1.33) | 0.761 | 0.82(0.67;1.01) | 0.063 | 0.82(0.54;1.25) | 0.358 | 0.87(0.72;1.05) | 0.140 |
| Z-score Height/age<2SD in cm/month, cm/kg, mean (SD) | 1.03(0.97 ;1.08) | 0.372 | 1.02(0.97;1.07) | 0.486 | 0.98(0.89;1.09) | 0.768 | 1.02(0.97;1.07) | 0.442 |
| Diarrhea | 1.84(1.22;2.78) | **0.003*** | 1.97(1.12;3.46) | **0.019*** | 3.97(1.12;14.02) | **0.032*** | 1.36(0.8;2.3) | 0.259 |
| Dehydration | 0.74(0.28;1.94) | 0.538 | 0.65(0.22;1.98) | 0.452 | 1.35(0.17;10.4) | 0.775 | 0.58(0.09;3.51) | 0.550 |
| Edema | 6.27(1.56;25.23) | **0.010*** | 6.04(1.38;26.39) | **0.017*** | 0.82(0.01;52.99) | 0.926 | 2.2(0.28;17.39) | 0.455 |
| Convulsion | 3.46(1.21;9.87) | **0.021*** | 2.06(0.6;7.03) | 0.248 | 0.68(0.02;29.4) | 0.844 | 2.34(0.5;11.01) | 0.281 |
| Axillary temperature [39.5,42] | 2.25(1.34 ;3.79) | **0.002*** | 3.01(1.51 ;5.97) | **0.002*** | 0.97(0.15 ;6.39) | 0.975 | 1.41(0.68 ;2.9) | 0.356 |
| Pallor of conjunctiva | 1.53(0.74;3.14) | 0.249 | 1.31(0.55;3.15) | 0.540 | 2.76(0.49;15.53) | 0.250 | 1.01(0.31;3.31) | 0.982 |
| Splenomegaly | 1.78(0.73;4.300) | 0.638 | 2.28(0.9;5.77) | 0.081 | 0.34(0.01;7.9) | 0.501 | 1.17(0.25;5.53) | 0.840 |
| Hepatomegaly | 1.17(0.50;2.75) | 0.725 | 1.03(0.38;2.78) | 0.951 | 0.25(0.01;5.15) | 0.372 | 0.79(0.18;3.37) | 0.746 |
| **Laboratory basic data**[†] | | | | | | | | |
| Hemoglobin <8 | 1.34(0.68;2.62) | 0.394 | 1.96(0.82;4.69) | 0.133 | 0.95(0.2;4.63) | 0.954 | 1.05(0.44;2.48) | 0.913 |

*: Statistically significant

[†]: hemoglobin value was g/dl; leucocyte value was $10^3/\mu l$

BSI: Bloodstream infection; GII: Gastro-intestinal infection; UTI: Urinary tract infection.

**Note:** The different data were adjusted for gender, age and weigh for the multivariate analysis.

The performance of clinical signs and symptoms significantly associated to bBSI were reported in table 4. Clinical signs and symptoms associated to bacterial BSI after multivariate analysis were combined to determine their performance to predict BSI in febrile children under-5 years. If we apply the combination "presence of diarrhea and edema" to determine whether an actual bacterial BSI is present, only one case of bacterial BSI out of 65 positive bacterial BSI (1.54%) detected by blood culture could be diagnosed. Three cases out of 65 positive bacterial BSI (4.62%) could be diagnosed if the combination "absence of diarrhea and presence of edema" was used.

**Table 4: Performance of significant clinical variables in multivariate analysis to predict BSI in febrile children under-5 years of age.**

| | | Blood culture positive n (%) | Blood culture negative n (%) |
|---|---|---|---|
| | | **Diarrhea+** | |
| **Diarrhea +** | Yes | 29(2.64) | 342(31.12) |
| | No | 36(3.27) | 692(62.97) |
| | | **Edema+** | |
| **Edema+** | Yes | 4(0.36) | 6(0.55) |
| | No | 61(5.55) | 1028(93.54) |
| | | **Diarrhea + edema +** | |
| **Diarrhea + edema +** | Yes | 1(0.09) | 0(0) |
| | No | 64(5.82) | 1034(94.09) |
| | | **Diarrhea - edema +** | |
| **Diarrhea - edema +** | Yes | 3(0.27) | 6(0.55) |
| | No | 62(5.64) | 1028(93.54) |
| | | **Diarrhea + edema -** | |
| **Diarrhea + edema -** | Yes | 28(2.55) | 342(31.12) |
| | No | 37(3.37) | 692(62.97) |
| | | **Diarrhea - edema -** | |
| **Diarrhea - edema -** | Yes | 33(3.00) | 686(62.42) |
| | No | 32(2.91) | 348(31.67) |

## Discussion

The present study showed that high fever (axillary temperature >39.5ºC), diarrhea and edema were only associated with a bacterial BSI in febrile children under-5 years of age. However, there is a risk to overlook a real bacterial BSI if only the clinical signs and symptoms combined to basic hematology data are used in the assessment of the child (only 1/65 case of BSI could be diagnosed if presence of edema and diarrhea should be considered). Although clinical signs and symptoms combined with basic hematological data are useful for suspicion of bacterial infections, there is a need to determine the presence of bacteria in clinical specimens related to the site of infection. Furthermore, there is a necessity to develop practical tools to distinguish bacterial infections from other infections in febrile children in order to be able to treat fatal, but treatable, diseases, in particular when a reliable diagnostic test would be available in an early stage of the infection or at the first contact with health professionals (21). Therefore, we

conclude on the basis of our data that clinical signs and symptoms, and basic hematology data cannot diagnose bacterial infections in febrile children under 5 years of age. As a consequence, this may lead to over prescription of antibiotics as attending health workers do not want to take the risk of missing a diagnosis. A definitive alternative to diagnose bacterial infections remains the development of practical laboratory tools, similar to malaria rapid diagnostic test, in other words cheap, fast and easy to perform without much training.

Previous study demonstrated that malaria may predispose to non-typhoid salmonella (NTS) bacteremia (22) (23). In the present study, malaria prevalence was 50% and *Salmonella enterica* (80.5% of positive blood culture and 50% of stool culture) was the main species isolated from blood and stool culture (18) (24) (25) (26). Therefore, NTS should always be considered in the case of a suspected bacterial infection in a malaria endemic area.

Studies by Mtove et *al*. (27) and Brent et *al*. (28) have both demonstrated a relative low positivity of blood stream infection in African children who do not meet indications for hospital admission. Conversely, other studies on blood stream bacterial infections in African children admitted to hospital have demonstrated that bloodstream or cerebral-spinal fluid (csf) bacterial infections are relatively common in children admitted to hospital and significantly influence mortality (29) (30). In our study, it was found that children who meet indications for hospital admission were more prevalent in the group of patients with bacterial infections than those without a bacterial infection. However, almost 70% of children with bacterial infections in general and 55% of children with positive bacterial BSI did not meet indications for hospital admission. Health workers should therefore pay more attention to children who did not meet indications for hospital admission at the first contact, in particular when a malaria infection can be excluded.

Our study confirms the limitation of using clinical signs and symptoms, and basic laboratory data to diagnose bacterial infections (31) (32). In general, only severe cases of bacterial infections are reported to be diagnosed by clinical signs and symptoms in young children (33) (34). In our study the combination of presence of diarrhea and edema (associate to BSI after multivariate analysis) to diagnose bBSI leaded to miss important part of bBSI. Nonetheless, high axillary temperature remains an indicator of bBSI (35) (36). Therefore, it is unlikely to save the lives of febrile children based on clinical signs and symptoms, and basic hematology data if bacterial diagnosis cannot be done at the early stage of infections. The availability of practical diagnostic tools for screening could be a good solution to save time and reduce the risk of fatal issues in the management of fever in this age group (21).

Although the multivariate analysis showed an association between UTI and diarrhea, the low prevalence of UTI deserve more attention. Previous studies reported association between UTI with high axillary temperature, sex and age (12–35 months) (37). However, urine sample collection deserves minimum hygiene condition. In the present study, stool and urine were systematically collected for each participant by parent/guardian and *E. coli* was the only specie isolated. Maybe the urine samples were contaminated with stool during urine sample collection.

Bacterial GII was not associated to any clinical signs and symptoms, and also basic hematology data. This is in line with the general observation that bacterial GII is not associated with fever (38) (39) (40) (41) (42).

A limitation of our study is that it does not present information on bacterial respiratory tract infections. The Pneumonia Etiology Research for Child Health (PERCH) project, a multi-country, case-control study to determine the etiology of and risk factors for (very) severe pneumonia in young children (1–59 months of age), provides a wealth of information on respiratory infection in African children (43). These studies have demonstrated that nasopharyngeal carriage of *S. pneumonia* and *S. aureus* is very common in healthy children (44) but their role as potential fever causing pathogen was not further studied here as such research would require a case-control design, which is beyond the scope of the present work.

**Conclusions**

Despite the usefulness of clinical signs and symptoms in combination with basic hematology data in detecting bacterial blood stream infections, our study demonstrated the necessity to confirm the presence of bacterial infection with practical diagnostic tools. Nevertheless, the worldwide concern about the over prescriptions of antibiotics cannot be circumvented if clinical signs and symptoms, combining to basic hematology data remain the only information available in area without laboratory facilities. The development of practical and easy deployable diagnostic tools to diagnose bacterial infections remains a priority.

**List of abbreviations**

API: Analytical Profile Index

BD: Becton Dickinson

BSI: Bloodstream Infection

CFU: colonies forming units

CG: Chocolate Gelose

CLED: Cysteine Lactose Electrolyte Deficient

CMA: Centre Médical avec Antenne Chirurgicale

$CO_2$: carbon dioxide

CRF: Case Report Form

CRUN: Clinical Research Unit of Nanoro

CSF: Cerebral-spinal fluid

EDTA: Ethylene-Diamine Tetra-Acetic acid

EMB: Eosin-Methylene Blue

EPI: Extended Program Immunization

GII: Gastro-Intestinal Infection

IVX: IsoVitalex

MUAC: Mid - Upper Arm Circumference

NICD: National Institute for Communicable Diseases

NPV: Negative Predictive Value

NTS: Non-Typhoid Salmonella

PERCH: Pneumonia Etiology Research for Child Health

PPV: Positive Predictive Value

RDT: Rapid Diagnostic Test

SB: Sheep Blood

SS: Salmonella and Shigella

UTI: Urinary Tract Infection

WHO: World Health Organization

**Declarations**

*Ethics approval and consent to participate*

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent was obtained from parents or guardians for the participation of the children prior to enrolment in the study.

*Consent for publication*

Not applicable.

*Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors declare that they have no competing interests

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*Authors' contributions*

FK, HS, PM, HT and MBvH conceived and designed the study. FK, MB and MT supervised patient inclusion, taking of informed consent and diagnostic specimen collection by study nurses. KF, PL, MT and MB performed the laboratory analyses. FK analyzed the data under the supervision of a biostatistician. FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript.

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# *Chapter 8*

## *Algorithms for sequential interpretation of a malaria rapid diagnostic test detecting two different targets of Plasmodium species to improve diagnostic accuracy in a rural setting (Nanoro, Burkina Faso)*

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**PLOS One: Accepted**

**Abstract**

**Background:** Malaria rapid diagnostic tests (RDT) have limitations due to the persistence of histidine-rich protein 2 (HRP2) antigen after treatment and low sensitivity of *Plasmodium* lactate dehydrogenase (*p*LDH) based RDTs. To improve the diagnosis of malaria in febrile children, two diagnostic algorithms, based on sequential interpretation of a malaria rapid diagnostic test detecting two different targets of *Plasmodium* species and followed by expert microscopy, were evaluated.

**Methods:** Two diagnostic algorithms were evaluated using 407 blood samples collected between April and October 2016 from febrile children and the diagnostic accuracy of both algorithms was determined. Algorithm 1: The result of line T1-HRP2 were read first; if negative, malaria infection was considered to be absent. If positive, confirmation was done with the line T2-*p*LDH. If T2-*p*LDH test was negative, the malaria diagnosis was considered as "inconclusive" and microscopy was performed; Algorithm 2: The result of line T2-*p*LDH were read first; if positive, malaria infection was considered to be present. If negative, confirmation was done with the line T1-HRP2. If T1-HRP2 was positive the malaria diagnosis was considered as "inconclusive" and microscopy was performed. In absence of malaria microscopy, a malaria infection was ruled out in children with an inconclusive diagnostic test result when previous antimalarial treatment was reported.

**Results:** For single interpretation, the sensitivity of *Pf*HRP2 was 98.4% and the specificity was 74.2%, and for the *p*LDH test the sensitivity was 89.3% and the specificity was 98.8%. Malaria was accurately diagnosed using both algorithms in 84.5% children. The algorithms with the two-line malaria RDT classified the test results into two groups: conclusive and inconclusive results. The diagnostic accuracy for conclusive results was 98.3% using diagnostic algorithm 1 and 98.6% using algorithm 2. The sensitivity and specificity for the conclusive results were 98.2% and 98.4% for algorithm 1, and 98.6% and 98.4% for algorithm 2, respectively. There were 63 (15.5%) children who had an "inconclusive" result for whom expert microscopy was needed. In children with inconclusive results (*Pf*HRP2+/*p*LDH- only) previous antimalarial treatment was reported in 16 children with malaria negative microscopy (16/40; 40%) and 1 child with malaria positive microscopy (1/23; 4.3%).

**Conclusion:** The strategy of sequential interpretation of two-line malaria RDT can improve the diagnosis of malaria. However, some cases will still require confirmative testing with microscopy or additional investigations on previous antimalarial treatment.

Key words: malaria, *Plasmodium falciparum*, diagnosis, rapid diagnostic test.

**Background**

Nowadays, malaria parasite specific antigen-detecting rapid diagnostic tests (RDTs) are considered essential tools in routine practice for the management of febrile illness in malaria endemic settings (1) (2) (3). The short time needed to obtain a diagnostic result (within 30 minutes), the simple read-out system and easy performance (no need for a conventional laboratory or extensive training), combined with relative low costs have contributed to the acceptance of this method in differentiating malaria fever from other causes of fever. The most commonly used RDTs in Africa, including Burkina Faso, are the *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) based RDT, which is specific for *Plasmodium falciparum* only, and the *Plasmodium* lactate dehydrogenase (*p*LDH) detecting RDT, which can detect 4 human *Plasmodium* species (*Plasmodium falciparum, P. ovale, P. malariae* and *P. vivax*) or a combination test, which has two test lines (a *P. falciparum*-specific line and an all human *Plasmodium*-test line).

In malaria endemic settings, several studies have been conducted to assess the sensitivity and specificity of *Pf*HRP2 and *p*LDH-based RDTs using microscopy as the gold standard (4) (5) (6) (7). Most of these studies reported a relative low specificity for *Pf*HRP2 detecting RDTs and low sensitivity for *p*LDH detecting tests (<95%). Consequently, *Pf*HRP2 is useful for active detection of new malaria infection but unsuitable for monitoring parasite clearance following antimalarial treatment due to the persistence of HRP2 antigen in the blood for up to four weeks and over, which is causing false positive *Pf*HRP2 results and information on prior drug use should always be collected (4) (5) (7) (8) (9) (10) (11). On the other hand, *p*LDH is metabolized within one week following antimalarial treatment and RDTs based on detection of this particular antigen were found to be suitable for monitoring parasite clearance, but less useful to detect new infections with a relatively low parasitaemia (12). As a consequence, a malaria positive *Pf*HRP2 based RDT may not signal also for another cause of fever and a malaria negative *p*LDH based RDT may not detect a low malaria parasitemia. So, the incidence of false positive *Pf*HRP2 and false negative *p*LDH tests has created a diagnostic dilemma, often resulting in a loss of trust and non-adherence of health workers for malaria RDT results in general (13).

To circumvent this dilemma, sequential reading/interpretation of the two-line RDT detecting HRP2 and *p*LDH, specific of *Plasmodium* species, has been proposed.

## Methods

### Study site

The study was performed in the health district of Nanoro, central-west of Burkina Faso. Samples and data were collected in three peripheral health facilities (Nanoro, Nazoanga and Seguedin), and in the referral hospital, the Centre Médical avec Antenne Chirurgicale (CMA) Saint Camille of Nanoro. The only diagnostic tool available for the routine diagnosis of fever in patients seeking heath care in these clinics is a malaria RDT. The RDTs used in government clinics are one based on the detection of *Pf*HRP2 (manufacturers: SD Bioline: Standard Diagnostics, Hagal-Dong, Korea; CareStart: Access Bio, Inc.). The management of uncomplicated medical cases, including uncomplicated malaria (positive malaria RDT), is done at the health facilities by nurses according to the national guideline of integrated management of childhood illness (14). Suspected malaria infection is the first reason for consultation in children under-5 years of age in this region and predominately occurs during the rainy season between July and November. The annual prevalence of malaria in children under-5 years of age has previously been determined to be 49.7% (15)

### Study design

This study was conducted in the framework of a large program aiming to improve the diagnosis and management of non-malaria fevers in children under-5 years of age (15). Briefly, all febrile children (axillary temperature ≥37.5°C) under-5 years of age presenting in one of the three health facilities or the referral hospital between April and October 2016 were invited to participate in the study. Non-malaria infections which can be a probable cause of fever, such as bacterial bloodstream infection, gastroenteritis infection and urinary tract infection, were also investigated in the framework of this large survey (15). Written informed consent was obtained from parent/guardian before any data or clinical specimen was collected. Basic demographic data of the included children and information on prior treatment, including antimalarials, in the two weeks before enrolment where collected.

For the purpose of the study a venous blood sample was collected in an Ethylene Diamine Tetra Acetic acid (EDTA) tube from each participant and specimens were transported to the Central Laboratory of the Clinical Research Unit of Nanoro (CRUN) for diagnostic (re-)testing (expert malaria microscopy and RDT's to detect *Pf*HRP2 and *p*LDH). Malaria RDTs were ordered, stored and transported as recommended by the manufacturer (17-30°C). The patients were managed according to the national guideline and malaria RDT provided by the National

Malaria Control Program (NMCP). The study was approved by the National Ethics Committee for Health Research of Burkina Faso (Deliberation N°2014-11-130).

**Laboratory procedure**

*Malaria rapid diagnostic test:*

A volume of 5 µl of a blood sample collected in an EDTA tube was used to perform the malaria RDT. A two-line malaria RDT detecting both *Pf*HRP2 and *p*LDH (SD Ag Bioline Malaria Ag *P.f/Pan*: Standard Diagnostics, Hagal-Dong, Korea; Lot number: 05EDC002A) with different lines specific for each target antigen and an internal control was used. The tests were performed and results recorded by a trained laboratory technician. A second trained technician also read and recorded the results of the RDT immediately after test execution and within the time limit set by the manufacturer. In case of discordance a third expert would be consulted to reach a decision. The test used in the present study went through the last WHO-FIND lot testing round 7 (Catalogue number: 05FK60) (16).

The performance of *Pf*HRP2 and *p*LDH were interpreted separately for the purpose of the study. In addition, the performance of the two-line malaria RDT was also calculated according to the manufacturer's instructions for the interpretation of the three lines malaria RDT (the test is positive if the at least one line of T1-HRP1 and T2-*p*LDH is positive and negative in case of absence of any positive line for the different targets).

To assess the performance of the proposed diagnostic algorithms including *Pf*HRP2 and *p*LDH, the three lines of the employed malaria RDT were used. The test was valid if the internal control line was positive. The line T1-HRP2 is specific to *Plasmodium falciparum* and the line T2-*p*LDH is positive when *P. falciparum*, *P. malariae*, *P. ovale* or *P. vivax* is present. For the purpose of the study the results of the specific HRP2-line or the specific *p*LDH-line where separately recorded on a case report form (CRF). The following two diagnostic algorithms were considered within the recommended time of the manufacturer:

Algorithm 1 and interpretation: Read line T1-HRP2 and confirmation with line T2-*p*LDH

For the interpretation for algorithm 1, the results of the line T1-HRP2 of blood samples tested form febrile children were read firstly: The absence of positive line T1-HRP2 indicates the absence of malaria infection and a confirmation with the line T2-*p*LDH is not required. However, the presence of a positive line for *Pf*HRP2 indicates the necessity to confirm by reading the line T2-*p*LDH testing. The presence of positive line T2-*p*LDH (next to the positive

line T1-HRP2) hence indicates the presence of malaria infection. The absence of a positive line T2-*p*LDH indicates that the diagnostic testing with RDTs was "inconclusive" and subsequently it was necessary to confirm the diagnosis with malaria expert microscopy (Fig 1).

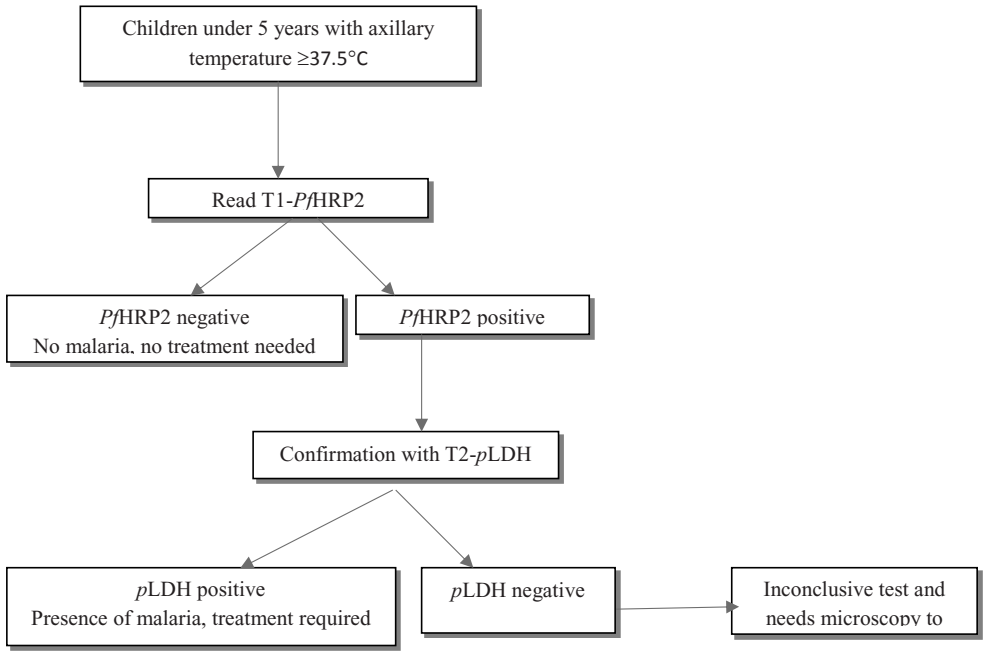


**Figure 1: Proposed algorithm 1 for the diagnosis of malaria: Read T1-*Pf*HRP2 first and confirm the T1-*Pf*HRP2 positive line results with T2-*p*LDH line.**

- Algorithm 2 and interpretation: Read line T2-*p*LDH and confirmed with the line T1-*Pf*HRP2

For the interpretation of algorithm 2, the results of the line T2-*p*LDH of blood samples tested from febrile children were read firstly: The presence of a positive line T2-*p*LDH indicates the presence of malaria infection and a confirmation with the line T1-HRP2 is not required. However, the absence of positive line T2-*p*LDH indicates the necessity to confirm with the line T1-HRP2. The absence of positive line T1-HRP2 (next to the absence of positive line T2-*p*LDH) indicates the absence of a malaria infection. The presence of positive line for T1-HRP2 indicates that the diagnostic testing with RDTs was "inconclusive" and that it was necessary to confirm the diagnosis with malaria microscopy (Fig 2).

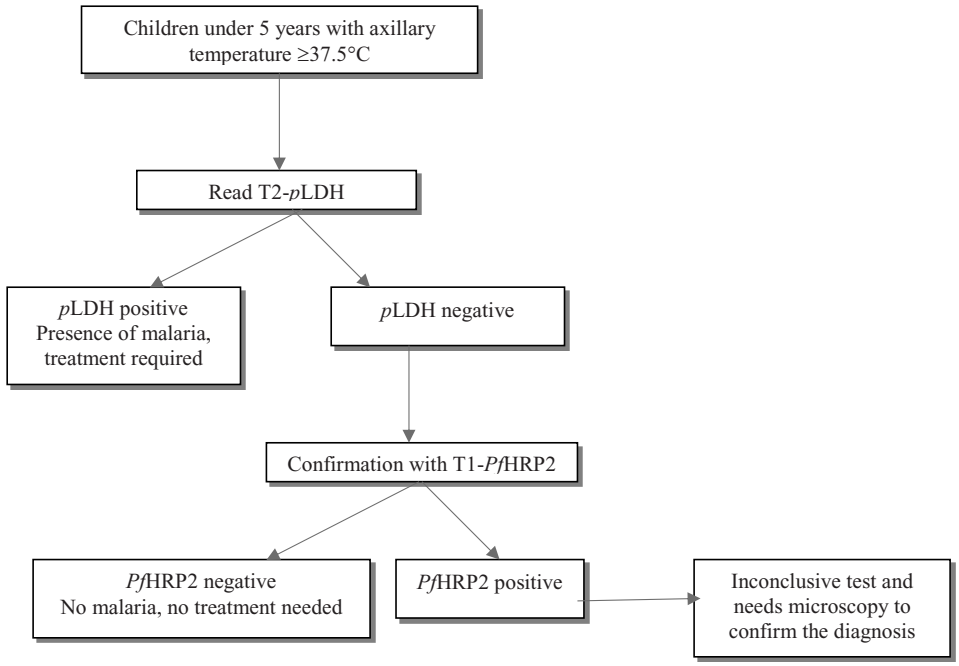


**Figure 2: Proposed algorithm 2 for the diagnosis of malaria: Read T2-*p*LDH and confirm the T2-*p*LDH negative line results with T1-*Pf*HRP2 line.**

If malaria microscopy to confirm the diagnosis in children with inconclusive diagnostic results is not available, additional information on previous antimalarial treatment within the past 2 weeks was used to exclude a malaria infection.

*Microscopy:*

Malaria diagnosis by microscopy was done by expert microscopists at CRUN who are subjected to regular external quality control (National Institute of Communicable Diseases/World Health Organization; NICD/WHO). At CRUN only accredited microscopists are authorized to read malaria slides (17). Thick and thin blood smears were prepared (in duplicate) from blood collected in EDTA tubes as previously described. Thick blood smears were considered negative when the examination of 100 field per thick film did not reveal the presence of any asexual parasites. Each blood slide was read and parasite densities counted by two independent readers and in case of discrepancy (positive vs negative, difference in *Plasmodium* species, difference in parasite density >Log10 or ratio >2 in case of parasite density ≤400 or >400/µl, respectively) by a third reader (15). These results were expressed as

asexual parasites per microliter by using the patient's white blood cells (WBC) count. Positive microscopy results were recorded as the geometric means of the two reader's results or the geometric means of the third reading and the closest reading of the two other readings. Microscopists were blinded from the malaria RDT results. A selection of slides (5%) was re-read by an independent expert microscopist for quality assurance.

Full blood counts

Full blood cell counts were done by using blood samples collected in EDTA tubes and a Sysmex XS1000i (Sysmex Corporation, Kobe, Japan) according to manufacturer's instructions.

Diagnosis of non-malaria infections

The methods applied for the diagnosis of non-malaria infections were in detail described previously (15). Blood and urine specimen were systematically collected for bacterial culture, and stools for rotavirus and adenovirus tests.

**Data analysis**

Data was entered in Excel 2016. Statistical analysis was performed by using R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For the purpose of this study, malaria microscopy was considered as gold standard. Sensitivity, specificity and predictive values were calculated for *Pf*HRP2, *p*PDH, the two-line malaria RDT (*Pf*/Pan) as recommended by the manufacturer and the two algorithms proposed. Proportion was used to describe qualitative data. For the quantitative data median was used to perform the description. Wilcoxon Rank Sum test was used to compare the median by estimating the p-value ($p \leq 0.005$) as statistically significant. Agreement between diagnostic approaches was determined by calculating Kappa (κ) values with 95% confidence intervals by using GraphPad software (https://www.graphpad.com/quickcalcs/).

**Results**

Characteristics of the study population

A total of 407 children under 5 years of age with documented fever were included in the study (Table 1). Of these children, 56.8% (231/407) were males and 25.6% (104/407) was under the age of 12 months. *Plasmodium falciparum* was found by expert microscopy in 60.0% (244/407) of the blood slides, and 5 *falciparum* patients were co-infected with other malaria

species (four with *P. malariae* and one with *P. ovalae*). The median parasite density was 39,847 (Interquartile range [IQR]: 7,828-95,369).

**Table 1: Characteristics of the study population (children under the age of 5 years with an axillary temperature of >37.5° C)**

| Characteristic | n (%) |
|---|---|
| **Gender** | |
| **Male** | 231 (56.8) |
| **Female** | 176 (43.2) |
| **Age (months)** | |
| **≤12** | 104 (25.6) |
| **>12** | 303 (74.4) |
| **Malaria prevalence (expert microscopy)** | 244 (60.0) |

Performance of two-line malaria RDT detecting PfHRP2/pLDH (P.f/Pan) compared to expert microscopy

Expert microscopy was, for the purpose of this study, considered as the gold standard. Based on the manufacturer's instructions, the *P.f/Pan*-based RDT was able to accurately diagnose 362 cases: i.e. 241 true positive cases (59.2%) and 121 true negative cases (29.7%). However, the *P.f/Pan*-based RDT had 42 (10.3%) false positive results and 3 (0.7%) false negative results (Table 2). The performance of *Pf*HRP2 and *p*LDH are also reported on table 2.

**Table 2: Diagnostic performance of *Pf*HRP2, *p*LDH and *Pf*/Pan compared with expert microscopy (gold standard) for the diagnosis of *Plasmodium falciparum* malaria.**

| | True positive | True negative | False positive | False negative |
|---|---|---|---|---|
| ***Pf*HRP2** | 240 (59.0) | 121 (29.7) | 42 (10.3) | 4 (1.0) |
| ***p*LDH** | 218 (53.6) | 161 (39.5) | 2 (0.5) | 26 (6.4) |
| ***Pf*/Pan** | 241 (59.2) | 121 (29.7) | 42 (10.3) | 3 (0.7) |

*P.f/Pan* = two-line malaria RDT detecting *Pf*HRP2 and *p*LDH; *Pf*HRP2 = *Plasmodium falciparum* specific histidine-rich protein 2; *p*LDH = *Plasmodium* lactate dehydrogenase.

The diagnostic accuracy of the *P.f/Pan*-based RDT results compared to expert microscopy as gold standard are presented in table 3. The sensitivity, specificity, positive predictive value and negative predictive value were 98.8% (241/244), 74.2% (121/163), 85.2% (241/283) and 97.6% (121/125) respectively. The agreement between *Pf*HRP2 testing and expert microscopy was considered to be "good; kappa value = 0.760 (95% CI 0.696 to 0.825; SE of kappa = 0.033). The sensitivity, specificity, positive predictive value and negative predictive value were respectively 98.4%, 74.2%, 85.1% and 96.8% for *Pf*HRP2 target and 89.3%, 98.8%, 99.1% and 86.1% for *p*LDH target (Table 3).

**Table 3: Diagnostic accuracy of *Pf*HRP2, *p*LDH and *P.f/Pan* compared with expert microscopy (gold standard) for the diagnosis of *Plasmodium falciparum* malaria.**

| | Sensitivity | Specificity | PPV | NPN |
|---|---|---|---|---|
| ***Pf*HRP2** | 98.4 (95.9-99.5) | 74.2(66.8-80.8) | 85.1(81.5-88.1) | 96.8(91.9-98.8) |
| ***p*LDH** | 89.3(84.8-92.9) | 98.8(95.6-99.8) | 99.1(96.5-99.8) | 86.1(81.1-89.9) |
| ***Pf*/Pan** | 98.8(96.3-99.8) | 74.2(67.0-80.4) | 85.2(80.5-88.86) | 97.6(92.8-99.5) |

*P.f/Pan* = two-line malaria RDT detecting *Pf*HRP2 and *p*LDH; PPV= positive predictive value; NPV= negative predictive value.

Diagnostic performance of algorithm 1 and 2

The diagnostic performance of algorithm 1 and 2 was comparable (Table 4). Algorithm 1 (read firstly the line T1-HRP2, followed by confirmation with the line T2-*p*LDH) resulted in 344 RDT confirmed diagnostic results: 219 positive cases and 125 negative cases. There were 63 "inconclusive" results (15.5% of all cases) that would require a final confirmation with microscopy if this algorithm would be applied. Algorithm 2 (read firstly the line T2-*p*LDH, confirmation with the line T1-HRP2) resulted also in 63 "inconclusive" results, and 220 positive and 124 negative cases. The algorithm 1 and 2 classified the diagnostic results in two groups: the conclusive diagnostic results in 84.5% (344/407) of children tested for which any additional information is not required to confirm malaria infection and the inconclusive diagnostic results in 15.5% (63/407) of children tested for which additional investigation is required following the algorithms. The conclusive results had higher median of parasites density of 45746 parasites/µl (IQR: 16618-101972) compared with inconclusive results with median of parasite density 778 parasites/µl (IQR: 119-1059.75) ($p<0.0001$).

**Table 4: Summary of the diagnostic performance of algorithm 1 and 2.**

| | Screening | | Confirmation | | Results of the applied algorithm | | |
|---|---|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | Positive | Negative | Inconclusive** |
| **Algorithm 1** | 282* | 125 | 219 | - | 219 | 125 | 63 |
| **Algorithm 2** | 220 | 187* | - | 124 | 220 | 124 | 63 |

*Needs to be confirmed by the second line of the two-line malaria RDT.
**Needs confirmation with malaria microscopy as the result of the two rounds of sequential read/interpretation with the two-line malaria RDTs is inconclusive.

There were thus 63 "inconclusive" test results with either employed diagnostic algorithm. For a final diagnostic result, these cases (15.5% of the whole study population) should be retested with expert microscopy. In the case of algorithm 1 and 2, these "inconclusive" test results comprised 23 cases that were found positive with expert microscopy and 40 cases that were reported as negative. Previous antimalarial treatments were collected within 2 weeks preceding inclusion. Despite the limit (HRP2 antigens can persist over 4 weeks), in children with

inconclusive test (*Pf*HRP2+/*p*LDH- only), previous antimalarial treatments were reported in 16 children with malaria negative microscopy (16/40; 40%) and 1 child with malaria positive microscopy (1/23; 4.3%). Moreover, non-malaria infections (bacterial bloodstream infection, viral gastroenteritis infection and urinary tract infection) were diagnosed in 10 children with malaria negative microscopy (10/40; 25%) and 2 children with malaria positive microscopy (2/12; 8.7%; parasite density of 32 and 78 parasites/µl).

The agreement between expert microscopy and the two diagnostic algorithms on the 344 conclusive test results (i.e. those that were not requiring confirmation with microscopy) is summarized in Table 5. The frequency of true positives was similar in algorithm 1 and 2 (63.1% and 63.4% respectively) as well as the frequency of true negatives (35.2% for the both). Furthermore, the frequency of malaria false positives was similar in algorithm 1 and 2 (0.6%) as well as the frequency of false negatives (1.2% versus 0.9% respectively). The agreement between expert microscopy and diagnostic algorithm 1 (using the results of the "conclusive" RDT test results only) was "very good" (Kappa = 0.962; 95% CI 0.932 to 0.992; SE of kappa = 0.015). Furthermore, the agreement between diagnostic algorithm 2 and expert microscopy was in this case also "very good" (Kappa = 0.968; 95%CI 0.941 to 0.996; SE of kappa = 0.014).

**Table 5: Agreement between expert malaria microscopy and the two algorithms on the 344 conclusive test results. Algorithm 1 is: first reading HRP2-line and subsequent confirmation with a *p*LDH-line; Algorithm 2 is: reading with a *p*LDH-line and confirmation with a HRP2-line.**

| | True positive | True negative | False positive | False negative |
|---|---|---|---|---|
| **Algorithm 1** | 217 (63.1) | 121 (35.2) | 2 (0.6) | 4 (1.2) |
| **Algorithm 2** | 218 (63.4) | 121 (35.2) | 2 (0.6) | 3 (0.9) |

For the 344 "conclusive" diagnostic RDT results, the sensitivities, specificities, positive predictive values and negative predictive values were similar for algorithm 1 and 2 (Table 6). These values were 98.2% (217/221), 98.4% (121/123), 99.1% (217/219) and 96.8% (121/125) for algorithm 1, and for algorithm 2, 98.6% (218/221), 98.4% (121/123), 99.1% (218/220) and 97.6% (121/124).

When prior antimalarial treatment within the past 2 weeks was used to exclude malaria infection in children with inconclusive diagnostic result (hence considering them as being negative), the sensitivities, specificities, positive predictive values and negative predictive values calculated, were similar for algorithm 1 and 2. These values were 98.0%, 84.0%, 90.2% and 96.5% for algorithm 1 and 98.4%, 84.0%, 90.2% and 97.2% for algorithm 2, respectively.

Consequently, inadequate prescription of antimalarial observed with *Pf*HRP2-based RDT could be improved (26 cases when algorithms are used compared to 42 children when *Pf*HRP2-based RDT only is used). Moreover, malaria infection could be missed in 5 children when algorithms are used versus 4 children when *Pf*HRP2-based RDT is used [data not show].

**Table 6: Diagnostic accuracy of algorithm 1 or 2 compared with expert microscopy (gold standard) for the diagnostic of *Plasmodium falciparum malaria* of 344 conclusive diagnostic test results obtained with sequential reading of two-line malaria RDTs**

| | Sensitivity | Specificity | PPV | NPN |
|---|---|---|---|---|
| **Algorithm 1** | 98.2 (95.4-99.5) | 98.4 (94.3-99.8) | 99.1 (96.5-99.8) | 96.8 (92.0-98.8) |
| **Algorithm 2** | 98.6 (96.1-99.7) | 98.4 (94.3-99.8) | 99.1 (96.5-99.8) | 97.6 (92.9-99.2) |

PPV= positive predictive value; NPV= negative predictive value.

## Discussion

The results of two diagnostic algorithms under evaluation demonstrated that the true and false results, which could not be diagnosed with single malaria RDT, could now be diagnosed and the test result can be classified in two groups: (i) the conclusive results for which malaria was diagnosed with a higher accuracy (>98%) following the algorithms; (ii) the inconclusive results for which malaria microscopy or additional investigation is required to better diagnose malaria in this group. The high sensitivity and specificity (>98%) of the algorithms for conclusive results only proofs that potential false positive and negative results could be identified by healthcare workers in a primary healthcare setting to improve the diagnosis of an ongoing infection. Consequently, healthcare workers are better able to accurately diagnose malaria in this group. This study also reported an association between the inconclusive results and the low malaria parasitemia, which is in line with other studies that reported that the low sensitivity of *p*LDH and the high sensitivity of *Pf*HRP2 leads to malaria false negative and positive results respectively. Moreover, after successful antimalarial treatments, *p*LDH antigen is metabolised within one week, but HRP2 antigens can persist in the blood up to 4 weeks and over. It is evident that low malaria parasitemia and persistence of HRP2 antigen after successful antimalarial treatment could lead to inconclusive diagnostic results (HRP2+/*p*LDH-) following the algorithms. A previous study reported that in a malaria endemic area, almost 50% of non-malaria infection due to bacteria and virus can be hide under a false positive HRP2 results (15). Based on this observation, it is obvious that inconclusive diagnostic result reported in the present study is due either to low parasitemia or non-malaria infection occurred after a successful antimalarial treatment.

The association between false positive HRP2 results and previous antimalarial treatment within the past 4 weeks (or more) has been reported by Maltha et *al*. and Dalrymple et *al*. (4) (10). It is therefore obvious that in absence of malaria microscopy, additional information on previous antimalarial treatment could be used to further establish the actual cause of fever in children with inconclusive test results. In the present study, information on previous antimalarial treatment partly resolved inconclusive diagnostic results, this is however a limited solution in the present study as only information on previous use in the last 2 weeks (and not 4 weeks) was collected. .In routine practice, healthcare workers should firmly ask for information on previous malaria treatment within the past 4 weeks to further establish the actual cause of fever in children with inconclusive test results.

Despite the limit in the collection of information on previous antimalarial treatments to resolve the inconclusive results, the diagnostic accuracies for the algorithms were higher than either test band individually reported in the present study and those reported by Hawkes et *al*. (sensitivity=88%; specificity=82%) (12). Consequently, the sequential interpretation of a two-line malaria RDT detecting both *Pf*HRP2 and *p*LDH could improve the diagnostic accuracy of malaria infection if the sequential interpretation proposed is supported by information on previous antimalarial treatment within the last 4 weeks in children with inconclusive results. In other words, the limitations of separately interpreting *Pf*HRP2 RDT, *p*LDH RDT or two-line malaria RDT detecting *Pf*HRP2 and *p*LDH can be greatly avoided. The sequential interpretation of the two-line malaria RDT detecting *Pf*HRP2/*p*LDH, supported by information on previous antimalarial treatment could improve the diagnostic accuracy. This approach can improve the management of febrile cases. The algorithms evaluated provided reliable alternatives to circumvent the diagnostic dilemma created by the use of a single malaria RDT. Malaria was accurately diagnosed with a diagnostic accuracy of 98.3% using diagnostic algorithm 1 or 98.6% with diagnostic algorithm 2. There were cases of febrile children (15.5%) in this study who had an "inconclusive" diagnostic test result and for whom expert malaria microscopy or other additional diagnostic and clinical investigations would be needed in order to do not miss potential treatable etiologies, including malaria. This indicates that both algorithm 1 and 2 can be helpful to accurately diagnose malaria in the majority of febrile children (84.5%). Both approaches eliminate doubts on the reliability of the diagnostic results achieved with the RDTs and the subsequent risk of having a malaria false positive or negative test result. The algorithms are designed for the diagnosis of *Plasmodium falciparum* only in area where *Pf*HRP2-based RDT are currently used. It is evident that the presence of non-

*falciparum* species, such as *Plasmodium vivax*, could impact the interpretation of the algorithms.

The number of malaria diagnostics results that were considered "conclusive" in our study with either algorithm was higher (84.5%) than that reported by Murungi et *al* (65.9%) (18) who followed a similar approach. Although the more sensitive polymerase chain reaction testing was not performed in our study, the sensitivity and specificity of the malaria diagnostic testing reported by Murungi for children under – 5 years was almost similar to that reported in our study on conclusive diagnostic test results; i.e. 100% for Murungi versus 98% in our study. In our study, 4 malaria microscopy positive cases (2 *Plasmodium falciparum,* 2 other *Plasmodium* species) were missed by our approach. Furthermore, there were two cases reported positive by our RDT approach, but with expert microscopy no parasites were found in the blood slides.

A group of children (n = 63; 15.5%) remained undiagnosed as testing for malaria with the proposed algorithms did not result in a conclusive malaria diagnosis. These children were ill as they had fever and 23 of them had malaria infection confirmed by microscopy, prompting the need of additional testing if the RDT approach proposed in this study remains inconclusive. These cases were *Pf*HRP2+/*p*LDH- possibly due to previous anti-malarial treatment resulting in persisting HRP2 antigen (4) or the low sensitivity of *p*LDH which is likely not to detect often parasite level below 2,000 parasites/µl (19). In absence of malaria microscopy, healthcare workers should pay more attention to these children because these inconclusive tests could conceal either a true malaria infection or non-malaria infections. In primary healthcare settings, nurses are responsible for health care and trained to manage uncomplicated infections based on clinical signs and symptoms, except malaria. So, it is evident that the information on the previous antimalarial treatment and non-malaria infections collected (based on signs and symptoms) could be helpful for clinical decision. Finally, clinicians should always pay particular attention to those children who are confirmed negative for malaria but do show clinical signs (like fever) as they may have other treatable causes of fever, such as bacterial infections (15).

This study was conducted in a seasonal malaria transmission setting and most samples were collected during the rainy season (July-October) when malaria transmission is high. Fewer children were included during the dry season (April-June). The intensity of malaria transmission could therefore influence the choice of which diagnostic algorithm is employed, but this has not been studied. Another potential limitation of algorithm 1 and 2 is in settings were HRP2 deletions occur (20) (21) (22). In such a setting a HRP2-line will always be

negative, even if an infection with *P. falciparum* is present. Consequently, in malaria endemic regions where HRP2 deletions are well established, the implementation of the proposed algorithms could increase the number of inconclusive results and misdiagnose some cases of malaria infection.

Finally, most likely the implementation of the proposed algorithms would be by sequentially using two different RDTs. However, this approach can double the time needed to confirm the diagnosis of a malaria infection as the result of the second test needs to be available too, and thismay also require taking a second blood sample. Research is needed towards patient and health care provider acceptability of such an approach.

**Conclusion**

The strategy of sequential interpretation of two-line malaria RDT can improve the diagnosis of malaria by grouping the low malaria parasitemia and false HRP2 positive children in inconclusive test for which additional investigations on previous antimalarial treatments are required.

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**Declarations**

*Ethics approval and consent to participate*

The study protocol was reviewed and approved by the National Ethical Committee in Health Research, Burkina Faso (Deliberation N°2014-11-130). Written informed consent for the participation of the children was obtained from parents or legal guardians prior to enrolment in the study.

*Consent for publication*

Not applicable.

*Availability of data and material*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors declare that they have no competing interests

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*Authors' contributions*

FK, HS, PM, HT and MBvH conceived and designed the study. FK, MB and MT supervised patient inclusion, taking of informed consent and diagnostic specimen collection by study nurses. KF, MT and MB performed the laboratory analyses. FK analyzed the data under the supervision of a biostatistician. FK and HS drafted the manuscript and all authors commented on draft versions. All authors read and approved the final manuscript

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# *Chapter 9*

*Discussion/Conclusion*

Despite efforts to improve malaria control in sub-Saharan Africa (SSA), fever still represents an enormous burden for children under 5 years of age (1) (2). The introduction of malaria rapid diagnostic tests (RDTs) has improved the management of malaria in primary healthcare settings, but it has created a new disease group called "non-malaria infection," for which the management remains presumptive Indeed, in primary healthcare settings, when malaria RDTs show a negative result, healthcare workers do not know which infection they should treat. Moreover, the low performance of the most common malaria RDTs used in SSA, based on *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) or on *Plasmodium* lactate dehydrogenase (*p*LDH), as reported in the field, might in fact jeopardize their primary aim (3) (4) (5) (6). Despite these doubts, the real value of malaria RDTs in the health system in SSA, including Burkina Faso, has rarely been assessed.

Each chapter of this thesis examines a different aspect of either the probable etiologies of fever episodes in children under 5 years or the value of RDTs in the management of these fever episodes in the routine system in primary healthcare centers.

**Etiologies of fever episodes in children under 5 years of age**

Infectious diseases remain the main cause of mortality and morbidity of young children in developing countries (7) (8) (9) (10) (11) (12) (13) (14) (15). The burden of mortality and morbidity attributable to these infectious diseases, in terms of disability adjusted life years (DALYs), is higher when compared to developed countries. For example, the DALYs for low respiratory tract infections are $292.1 \times 10^5$ for LMICs and $1.73 \times 10^5$ for high income countries (8) (16) (17) (18) (19). Fever may be caused by parasitic, bacterial or viral infections, with a geographical distribution which may depend on climate (temperature, wind, humidity), population and physiopathology factors (20) (21) (22). The epidemiological surveillance of infectious diseases is therefore vital in order to be able to develop a group of priority diseases of public health importance.

Many initiatives to eradicate malaria – one of the most significant infectious diseases epidemiologically speaking – have been undertaken in SSA (23). Despite the global decline of malaria morbidity and mortality (2) (24), the main finding of the study that is reported in **Chapter 3** is that malaria remains an important (possible) cause of fever in our study area, as is the case in many SSA countries (25) (26) (27) (28). Yet despite the World Health Organization's (WHO) recommendations for the management of malaria in endemic countries (i.e. to diagnose a malaria infection prior to the administration of any treatment), non-malaria

fevers remain largely undiagnosed and are often managed presumptively on the basis of general clinical signs and symptoms.

To get a better understanding of the contribution of non-malaria infections to fever episodes in children under 5 years of age, a systematic review of the causes of fever between January 1990 and July 2015 in SSA was performed (see **chapter 2**). This systematic review revealed that non-malaria fevers were infrequently studied in the years before the introduction of malaria RDTs, with only 5 papers published before 2010 (29) (30) (31) (32) (33) versus 13 papers published after 2010 (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46). In the pre-RDT era (before 2010), the real burden of non-malaria fevers was probably underestimated because fever episodes were mainly attributed to malaria infection, as the diagnosis was in principle based on clinical symptoms (i.e. fever = malaria) only. However, in **chapter 3**, it is shown that there is a very low proportion of co-infection between malaria-positive children and non-malaria infections, such as bacterial bloodstream infections (bBSIs), urinary tract infections (UTIs) and viral gastro-intestinal infections (vGII). This observation is confirmed in other studies in SSA that also determined the actual cause of fever in febrile children (47) (48) (49).

The etiologies of non-malaria fevers show considerable overlap between SSA countries, except for area-specific diseases associated with epidemic outbreaks such as Ebola and Lassa Fever. Among the febrile infections attributable to bacterial etiologies, bacterial bloodstream infections (bBSIs) and urinary tract infections (UTIs) were often found in children under 5 years. The most common bacteria responsible for bBSIs are *E. coli*, *typhoid*- and *non-typhoid Salmonella*. Less commonly found pathogens were Gram+ bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*). For UTIs, *E. coli* is the predominant bacteria (see **chapters 2 and 3**). The potential reservoirs of these pathogens are many: domestic and wild animals and sources of drinking water for *E. coli*, *typhoid* and *non-typhoid Salmonella* (50) (51) (52); and human for *Staphylococcus aureus* and *Streptococcus pneumoniae* (53). The lack of supervision of children under 5 years combined with limited hygiene conditions and sanitation might explain the high prevalence of these bacteria, as sometimes reported in SSA. Consequently, these infections are associated with poverty and can be partly prevented by acting on the socio-economic environment and behavior of the affected populations.

Although the prevention of infection is possible, the reality is that many febrile children have an infection that cannot be readily diagnosed at primary healthcare settings, such as bacterial and viral infections (54) (55) (56) (57) (58) (59), as is also the case, for example, with

*Streptococcus pneumoniae* (and rotavirus) observed in our study area (see **chapter 3**). Consequently, the management and treatment of children with fever is complicated and this situation is exacerbated by the fact that not all infectious diseases, such as viruses, require antibiotic treatment. Despite this, our study shows that many healthcare providers prescribe antibiotics irrespective of (whether they know) the actual cause of fever (see **chapter 6**).

**Performance of malaria RDTs in the field and the challenges of strict adherence of health workers to the test result**

Correct and timely diagnosis of infectious diseases remains a challenge in LMICs. Malaria microscopy is still the most commonly used method for diagnosing a *Plasmodium* infection, but it can only be performed by a qualified laboratory technician in a conventional laboratory (60) (61) (62) (63). This is even more true for molecular biology assays and microbiological diagnostics, which need very specific equipment and high-level trained laboratory personnel (64). Unfortunately, the implementation of these sophisticated methods is often hampered due to infrastructural, financial and capacity constraints in LMICs. There are currently no simple, practical tools available to diagnose non-malaria infections in remote primary healthcare settings. Therefore, the research presented in this thesis focused on the challenges of making a correct and timely diagnosis, and the effect of the malaria RDTs on the management of fever episode(s) in children under 5 years (**chapters 4, 5** and **6**).

The diagnostic accuracy of *Pf*HRP2-based RDTs is reported in **chapter 4**. A specificity issue was observed in this study when the test was performed by the nurses at the health facilities (52.8%: 95%CI: 44.8-60.6) compared to tests performed by a trained technician at the laboratory of the Clinical Research Unit of Nanoro (CRUN) (74.2%: 95%CI: 66.8-80.8). In particular, the relatively high number of false positives from malaria RDTs reported by the nurses working in the rural health facilities is worrying. Apart from an intrinsic low specificity of the test itself, incorrect test execution can also explain the false positive results observed at the health facility level. Some biological factors can also lead to a false positive reaction with an HRP2-based RDT (e.g. rheumatoid factors, hepatitis C, schistosomiasis, toxoplasmosis, dengue, leishmaniasis, Chagas disease and human African trypanosomiasis), but these interactions are considered to be rare (65) (66) (67) (68) (69) (70) (71) (72) (73) (74). Transport and storage conditions can also impact the performance of malaria RDTs in the field (75) (76) (77).

As the positive predictive value of malaria HRP2-based RDTs varies, its use to screen patients to differentiate malaria fever from non-malaria fever might have stopped health workers from considering alternative causes of fever. In the present study, almost 50% of patients with other causes of fever were diagnosed by the HRP2-based RDTs as malaria false positives. Cultures of clinical specimens from these patients confirmed the presence of a bacterial infection, whilst microscopy did not find any presence of malaria parasites. More importantly, all of the infections that were missed in the *Pf*HRP2 false positive group were treatable infections that could have caused severe disease and even fatality (**chapters 3** and **5**). The use of malaria RDTs to reduce the unnecessary prescription of antimalarials by healthcare workers may not be useful in the management of non-malaria etiologies of fever in countries where malaria and non-malaria etiologies coexist. The tests can give an incorrect feeling of confidence in the obtained diagnosis and may result in health workers not looking for other causes of diseases, as reported in **chapter 6**.

In addition, this thesis has shown that no association was found between the main causes of fever diagnosed by healthcare workers (based on malaria RDTs and presumptive diagnosis, such as respiratory tract, gastro-intestinal or urinary tract infections) and the antimicrobials that were actually prescribed (**chapter 6**). It was found that the prescriptions of antimalarials and antibiotics seems to be independent of the malaria HRP2 results. In other words, next to antimalarials, antibiotics were usually prescribed to children with malaria positive RDTs, presumably to also treat a possible bacterial infection present in a malaria positive case (**chapter 5**). Our sensitivity analysis shows that the prescription of antibiotics is influenced by the limitations of healthcare workers to correctly diagnose non-malaria diseases when malaria is ruled out. As expected, antimalarials were less frequently prescribed to children with malaria negative RDTs compared to children with malaria positive RDTs. Consequently, although malaria was most often diagnosed, for infections in febrile children in primary healthcare settings antibiotics remained the most commonly prescribed antimicrobial. It appears obvious that this prescription of antibiotics is likely due to a fear of overlooking possible bacterial etiology and sending sick children home untreated. However, the results of the malaria RDTs should not influence the doctors' decision to treat a possible bacterial infection.

The sequential interpretation of a two-step malaria RDT, as reported in **chapter 8,** could be a way to improve the accuracy of diagnosing malaria in rural settings. However, this does not reduce the urgent need for other practical and simple tools for the screening of other potential causes of fever in children.

Importantly, the good adherence rate (92.89%) of healthcare workers to the recommendations of the WHO for malaria management in our study area (**chapter 6**) implies that it is likely that health workers will also adhere to the outcome of other RDTs, if available (78) (79) (80).

**Barrier to clinical diagnosis and innovations in evidence-based diagnosis**

The study we conducted on the association between clinical signs and symptoms and basic hematology data with bacterial infections in febrile children, as described in **chapter 7**, showed that high fever (axillary temperature >39.5ºC), diarrhea and edema were associated with bBSIs. In this study, diarrhea was highly prevalent in children under 5 years (37.8%), as in other countries in SSA (81) (82). However, edema was less prevalent (0.9%) and could be associated with malnutrition in children under 5 years (83). Despite the role of clinical signs and symptoms in the diagnosis of severe bacterial infections in inpatient children (84) (85), there is a need to determine the presence of bacteria in clinical specimens related to the site of infection. Furthermore, there is a necessity to develop practical tools to support the clinical suspicion of bacterial infections in febrile children in primary healthcare settings.

There remains a need to improve malaria diagnosis, in particular to circumvent the issue of false positive RDTs. Two algorithms for the sequential interpretation of febrile patients with malaria RDTs detecting two different targets (*Pf*HRP2 and *p*LDH) were assessed in **chapter 8**. These algorithms provide reliable alternatives to circumvent the diagnostic dilemma created by the use of single malaria RDTs. Indeed, malaria could be accurately diagnosed in around 85% of febrile children (diagnostic accuracy >98%). However, some cases with inconclusive results (15.5%) still needed confirmation with malaria microscopy (86) (87). In the absence of malaria confirmation by microscopy, healthcare workers should pay more attention to these children in order not to miss other causes of fever. Importantly, in children with inconclusive results (HRP2+/*p*LDH-), malaria negative microscopies were more frequently reported among children with evidence of previous antimalarial treatment, despite the limits of the collected information. In the absence of malaria microscopy, the diagnosis of malaria in children with inconclusive results could be improved if the interpretation of the RDTs is supported by information on previous antimalarial treatment within the last 4 weeks.

With the decline of malaria incidence and the introduction of malaria RDTs in routine practice, it has become apparent that new tools need to be developed to efficiently diagnose other causes of fever. Many diagnostic tests available for the diagnosis of other causes of fever have

limitations in terms of sensitivity and/or specificity (88) (89). To circumvent these limitations, efforts to develop molecular-based diagnostics have increased over the last decade. Such a (diagnostic) platform could also be developed for the diagnosis of bacterial infections, in particular for bloodstream infections. Capitalizing on the successful management of malaria and human immunodeficiency virus (HIV) due to the deployment of RDTs (90) (91), the introduction of diagnostic kits for the rapid diagnosis of other infections will create an opportunity to improve the management of febrile diseases in rural areas. Before these bacterial RDTs can be recommended in routine care in rural settings, their performance should be evaluated.

**Concluding remarks**

With the availability of malaria RDTs, it has become apparent that the real causes of (treatable) febrile illness have been greatly underestimated for many years due to the heavy burden of malaria in young children. Due to the limitations of malaria RDTs in terms of the correct diagnosis of malaria infections and the interpretation of clinical signs and symptoms to predict bacterial infections, healthcare workers do not feel confident with the results of RDT testing and tend to prescribe both antimalarials and antibiotics. This leads to the over-prescription of antimicrobials; and one of the main consequences of this is emerging drug resistance.

In order to improve the accuracy of diagnosing malaria infections in primary healthcare settings in SSA, it is crucial to ensure adequate training of the healthcare workers who perform the RDTs. In addition, periodic quality checks of the RDTs, performed at the health facility level, are important to ensure the reliability of the results of RDTs. Importantly, most of the etiologies reported are treatable or can be prevented by vaccination or acted upon by changes in lifestyle and behavior of the population. Whatever the method of prevention, the development of practical tools for the diagnosis of non-malaria fever is imperative for the control of these infections. Moreover, governmental and sub-regional monitoring tools should be developed to monitor the execution of RDTs, as they are rolled out and performed by healthcare workers, and to control the prescription practices at these healthcare centers.

The management of fever in many LMICs has been greatly improved by the introduction of malaria RDTs. However, an important part of the young febrile population still remains undiagnosed due to a lack of appropriate tests to determine the other possible causes of fever, as well as due to reported limitations of malaria RDTs. The present work has demonstrated that

"other causes of fever" are as important as malaria in a febrile pediatric population in Burkina Faso. The initial success of the WHO's "test and treat" strategy is now at risk, and the over-prescription of antimalarials and antibiotics, with the associated danger of drug resistance, is becoming increasingly common. Some new strategies have been proposed in this thesis, such as sequential interpretation of malaria RDTs or a combination of clinical signs and symptoms with basic hematology, to improve the diagnosis, management and treatment of febrile disease in young children in LMICs, but these are not sufficient to circumvent the problem altogether. The development of new diagnostic approaches and tools remains of utmost importance and is a clear challenge for the years to come.

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# *Chapter 10*

*Summary*

*Samenvatting*

*Résumé*

## Summary

Fever remains the first cause of medical consultation in children under 5 years of age in Burkina Faso. For many years, fever was presumptively managed as malaria. This presumptive management was extended to other possible causes of febrile illness only in the case that the fever persisted following treatment with antimalarials. With the adoption of the World Health Organization's (WHO) recommendation to perform a rapid diagnostic test (RDT) in every presumptive malaria case prior to antimalarial treatment, practice has changed and this has led to a more rational prescription of antimalarials.

Despite global efforts in the prevention, diagnosis and treatment of malaria, however, fever remains an important health issue in many rural settings. The lack of practical tools to identify the cause of non-malaria fever leads to the systematic prescription of antibiotics by community healthcare workers when malaria is excluded by RDTs. This problem is exacerbated when malaria RDTs perform below expected diagnostic accuracy, producing false negative or false positive test results. The aim of this thesis is to provide more insight into the probable (treatable) causes of fever in febrile young children (under 5 years of age) and to assess the value of RDTs and current diagnostic practices in primary healthcare settings in Nanoro, Burkina Faso.

In **chapter 2** of this thesis, the literature on the etiologies of non-malaria fever in children under 5 years in sub-Saharan Africa (SSA) from 1990 to 2015 was systematically reviewed. From this review, it appears that the real burden of non-malaria fever was underestimated in the pre-RDT era compared to the post-RDT era. Among the non-malaria infections investigated during these 25 years, bloodstream infections (BSIs) were the most common in young children, followed by urinary tract infections (UTIs) and respiratory tract infection (RTIs). The etiologies of fever episodes reported in BSIs and UTIs were mainly bacterial. The viral etiologies reported were mainly Human *influenzae* A and B (RTIs) and rotavirus/adenovirus (gastrointestinal infections). Viral infections can often not be adequately treated, but all other etiologies (bacterial or parasitic) are potentially well treatable and require a proper diagnosis followed by appropriate treatment.

A year-long survey on the treatable causes of fever among children under 5 years of age was conducted in four healthcare centers of the health district of Nanoro as well as the reference hospital for the district, and was reported on in **chapter 3**. In addition to malaria, non-malaria infections, including gastrointestinal infections, the common bacterial pathogens of the

nasopharynx, bacterial bloodstream infections (bBSIs) and UTIs, were also investigated. Among the non-malaria infections, the detected infections that were defined as "the probable cause of fever" in the recruited cases were bBSIs, viral gastrointestinal infections and UTIs. From this survey amongst 683 children, it appeared that malaria remained the primary cause of fever (49.7%). Non-malaria infections were found in 49.1% of febrile children, among which 10.7% could be "a probable cause of fever." Three percent of the malaria infected children were co-infected with non-malaria infections which could be considered a probable cause of fever. In contrast, in malaria microscopy negative children, 18% of the non-malaria infection found could be the probable cause of fever. The other probable causes of fever were significantly associated with malaria microscopy negative children compared to malaria microscopy positive children ($p<0.001$).

The study reported in **chapter 4** showed a huge difference in the specificity of the RDTs depending on the operator. Nurses performing the RDTs at the health facility level reached a specificity of 52.8%, while the technician performing the RDTs at the laboratory of the Clinical Research Unit of Nanoro (CRUN) reached a specificity of 74.2%. Indeed, the difference between the number of malaria false positive cases produced by RDTs at the healthcare centers and by the same brand of RDTs at the central laboratory of CRUN was statistically significant ($p=0.0005$). The persistence of the Histidine-rich protein 2 (HRP2) antigen after successful antimalarial treatments could not be the reason for the observed difference. Several factors could explain the high number of false positive cases reported using the malaria RDTs supplied by the Ministry of Health, most likely operator errors during testing and biological factors. Although less probable, the transport and storage conditions could also affect test performance. Healthcare workers should be adequately trained. Moreover, periodic monitoring of the execution of the malaria RDTs by healthcare workers and periodic quality checks of the RDTs at the heath facilities should be implemented.

A large proportion of children with a malaria positive RDT were, unexpectedly, children with a malaria negative microscopy result (**chapter 5**). Of these children, 25% showed that they harbored other (non-malarial) treatable causes of fever, which are being missed if only the result of the HRP2 RDT for malaria is followed. In the whole group of children examined in this study, 49% of those diagnosed with other causes of fever had had a malaria false positive RDT. Moreover, 36% of these cases of other causes of fever were diagnosed in children with a malaria true negative RDT and 15% in the malaria true positive RDT cases. Based on these findings, healthcare workers must be aware that if fever does not subside after treatment with

adequate antimalarials, the alternative cause(s) of fever must be considered. All of the infections missed in the malaria RDT false positive group were treatable and could have caused serious illness and even fatality.

As a result of the findings in **chapter 5**, one consequence of the use of an HRP2-based RDT could be the inappropriate prescription of antimicrobials. **Chapter 6** describes how, despite the good attitude of healthcare workers to adhere to the outcome of malaria RDT testing, the prescription of antimalarials is affected by the performance of the particular malaria RDT used in the study area. Even if a malaria infection was diagnosed by an RDT, there was a tendency to prescribe antibiotics next to antimalarials. Moreover, the prescription of antibiotics had become systematic when a malaria infection was excluded. It is obvious that antibiotics are being prescribed due to a fear of overlooking a bacterial infection and sending untreated sick children home, even if a viral infection could just as well be the cause of the fever. However, the results of malaria RDTs should not influence doctors' decision to treat every RDT negative child as if he/she has a bacterial infection.

Doctors are thus in great need of simple diagnostic guidelines to guide their clinical decisions. A clinical algorithm would be the easiest solution, as this does not require any sophisticated tools. To determine the association between a bacterial infection and clinical signs/symptoms and basic hematology, a univariate analysis was done, and only variables with a p-value of <0.1 were considered for multivariate analysis (**chapter 7)**. The multivariate logistical regression analysis revealed that high temperature (>39.5ºC), diarrhea and edema were associated with bacterial BSIs. However, this association is not sufficiently specific or sensitive to predict all true bacterial infections in febrile children under 5 years. In low and middle income countries (LMICs), guidelines for the presumptive management of fever episodes are usually followed. However, these guidelines are merely tools of orientation, and do not provide a diagnostic algorithm. Because of the low discriminatory power of clinical signs and symptoms, even when they are combined with simple hematology, there is an urgent need to develop practical tools to distinguish bacterial infections from viral infections in febrile children.

Given the limits of *Pf*HRP2 and *p*LDH in the diagnosis of *Plasmodium falciparum*, two diagnostic algorithms based on sequential interpretation of a two-step malaria RDT detecting both *Pf*HRP2 and *p*LDH were assessed (**chapter 8)**. The proposed algorithms revealed several advantages: (i) malaria was accurately diagnosed in 84.5% of febrile children (diagnostic accuracy >98%); (ii) children with inconclusive results, for which malaria microscopy or

complementary information was needed, were also identified (15.5%); (iii) in children with inconclusive results, evidence of previous antimalarial treatments within the last four weeks could suggest a non-malaria infection; (iv) the inappropriate prescription of antimalarials could be reduced. In other words, this study suggests that a positive or negative result for *both* HRP2 and *p*LDH bands are required to improve the diagnostic accuracy of malaria infection in malaria endemic areas in 84.5% of febrile children. The study suggested that for children with inconclusive results (HRP2+/*p*LDH-), and in the absence of malaria microscopy, healthcare workers could consider adding information on previous antimalarial treatment within the past 4 weeks. If information on previous antimalarial treatment can be collected for this group, healthcare workers should take into account the fact that in children with evidence of antimalarial treatment within 4 weeks, the actual cause of fever cannot be malaria. Non-malaria infections should therefore be established.

This thesis illustrates that the actual causes of fever have been underestimated for many years due to the dominance of malaria in (the diagnosis of) young children. In many rural settings in SSA, a malaria RDT is the only diagnostic tool available to establish the cause of fever. Due to the limitations of these RDTs to correctly diagnose a malaria infection, antimalarials and antibiotics are inappropriately prescribed. To fill this gap in the diagnosis of malaria, a two-line malaria RDT could be introduced to achieve a more accurate diagnosis. Moreover, building further on the successful implementation of RDTs for the diagnosis of malaria and human immunodeficiency virus (HIV), the introduction of extended diagnostic kits for the rapid diagnosis of other common causes of fever should be considered, which would create opportunities to improve the management of febrile diseases in rural areas.

## Samenvatting

Koorts is bij kinderen onder de leeftijd van 5 jaar de voornaamste reden voor een medisch consult in Burkina Faso. Jarenlang werd koorts altijd als een vermoedelijke malaria behandeld. Andere mogelijke oorzaken van koorts werden alleen overwogen als de koorts aanhield na behandeling met anti-malaria middelen. Door aanbeveling van de Wereldgezondheidsorganisatie (WHO) te volgen om een snelle diagnostische (malaria) test (RDT) uit te voeren bij elk vermoedelijk geval van malaria voorafgaand aan de anti-malaria behandeling, is de praktijk veranderd en dit heeft geleid tot een meer rationeel voorschrijven van anti-malaria middelen.

Ondanks wereldwijde inspanningen in de preventie, diagnose en behandeling van malaria, blijft koorts een belangrijk gezondheidsprobleem in veel rurale gebieden. Het gebrek aan praktische hulpmiddelen om de oorzaak van koorts, niet veroorzaakt door malaria, te identificeren, leidt tot het systematisch voorschrijven van antibiotica door lokale gezondheidswerkers wanneer malaria via een RDT is uitgesloten. Dit probleem wordt verergerd wanneer de gebruikte malaria-RDT's beneden de verwachte diagnostische nauwkeurigheid presteren, waardoor vals-negatieve of vals-positieve testresultaten frequent voorkomen. Het doel van dit proefschrift is om meer inzicht te krijgen in de waarschijnlijke (behandelbare) oorzaken van koorts bij jonge kinderen (jonger dan 5 jaar) en om de diagnostische waarde van RDT's en de huidige diagnostische praktijken in de primaire gezondheidszorg in Nanoro, Burkina Faso, te beoordelen.

**Hoofdstuk 2** van dit proefschrift bevat een systematische review van de literatuur over de etiologie van niet-malaria koorts bij kinderen jonger dan 5 jaar in sub-Sahara Afrika (SSA). Uit dit review blijkt dat de feitelijke incidentie van niet-malaria koorts in het pre-RDT-tijdperk werd onderschat in vergelijking met het RDT-tijdperk. Van de niet-malaria infecties die gedurende 25 jaar werden onderzocht, waren bloedbaaninfecties (BBIs) het meest voorkomend bij jonge kinderen, gevolgd door urineweginfecties (UWIs) en luchtweginfecties (LWIs). De veroorzakers van koortsepisoden gerapporteerd in BBIs en UWIs waren voornamelijk bacterieel. De gerapporteerde virale veroorzakers waren hoofdzakelijk humaan influenza A en B (LWIs) en rotavirus/adenovirus (gastro-intestinale infecties). Voor de meeste virale infecties bestaat geen behandeling, maar de meeste bacterieel en parasitaire infecties zijn goed te behandelen, maar vereisen een juiste diagnose.

In **hoofdstuk 3** word een onderzoek naar de behandelbare oorzaken van koorts bij kinderen

jonger dan 5 jaar uitgevoerd in vier zorgcentra en het referentieziekenhuis van het gezondheidsdistrict Nanoro gepresenteerd. Uit dit onderzoek onder 683 kinderen bleek dat malaria de primaire oorzaak van koorts was (49,7%). Niet-malaria-infecties werden gevonden bij 49,1% van de kinderen met koorts, waarvan 10,7% "een waarschijnlijke oorzaak van koorts" kon zijn. Drie procent van de malaria-geïnfecteerde kinderen was gelijktijdig geïnfecteerd met niet-malaria-infecties die ook als een waarschijnlijke oorzaak van de koorts kunnen worden beschouwd. De andere "waarschijnlijke oorzaken van koorts" waren significant geassocieerd met malaria microscopie negatieve uitslagen ($p < 0,001$).

De studie beschreven in **hoofdstuk 4** toonde een enorm verschil aan in de specificiteit van de RDT's, welke afhankelijk was van degene die de test uitvoerde. Verpleegkundigen die de RDT's uitvoerden in de lokale gezondheidscentra bereikten een specificiteit van 52,8%, terwijl de analisten die de RDT's in het laboratorium van de Clinical Research Unit van Nanoro (CRUN) uitvoerden een specificiteit van 74,2% bereikten. Het verschil tussen het aantal vals-positieve malaria gevallen gevonden met RDT's in de gezondheidscentra en in het centrale laboratorium van CRUN met hetzelfde merk RDT was inderdaad statistisch significant ($p = 0,0005$). De persistentie van het Histidine-rijke eiwit 2 (HRP2)-antigeen na succesvolle anti-malaria behandelingen zou niet de reden voor het waargenomen verschil kunnen zijn. Verschillende factoren kunnen het hoge aantal fout-positieve gevallen verklaren die zijn gerapporteerd met de malaria-RDT's die zijn verstrekt door het ministerie van Volksgezondheid. De meest waarschijnlijke oorzaken zijn fouten gemaakt door de uitvoerders tijdens het doen van de testen en biologische factoren. Hoewel minder waarschijnlijk, kunnen ook de transport- en opslagomstandigheden de testprestaties beïnvloeden. Gezondheidswerkers moeten daarom voldoende worden getraind in het uitvoeren van de RDTs. Bovendien moet de uitvoering van de malaria RDT's door gezondheidswerkers regelmatig worden gecontroleerd en moeten er periodieke kwaliteitscontroles van de RDT's op de gezondheidsfaciliteiten plaats vinden.

Een belangrijk deel van de kinderen met een malaria-positieve RDT (HRP2) bleken, onverwacht, bij controle een negatieve uitslag te hebben bij de microscopie (**hoofdstuk 5**). Van deze kinderen had 25% een andere (niet-malaria) behandelbare oorzaak van koorts, welke zou zijn gemist als alleen het resultaat van de HRP2 RDT voor malaria zou zijn gevolgd. In de hele groep kinderen die in deze studie werd onderzocht, werd 49% van de andere oorzaken van koorts gediagnosticeerd bij kinderen met een malaria fout-positieve RDT. Bovendien werd in 36% van deze gevallen andere oorzaken van koorts gediagnosticeerd bij kinderen met een

malaria negatieve RDT en in 15% van de gevallen met een malaria-positieve RDT. Deze bevindingen geven aan dat zorgverleners zich ervan bewust moeten zijn dat als de koorts na behandeling met goede anti-malaria middelen niet weg gaat, alternatieve oorza(a)k(en) van koorts overwogen moeten worden. Alle gemiste infecties in de malaria RDT-foutpositieve groep waren te behandelen en hadden een ernstige ziekte en zelfs de dood kunnen veroorzaken. **Hoofdstuk 5** laat zien dat een gevolg van het gebruik van een op HRP2 gebaseerde RDT onjuist voorschrijven van antimicrobiële middelen kan zijn. **Hoofdstuk 6** beschrijft hoe, ondanks de goede houding van gezondheidswerkers om zich aan de uitkomst van malaria-RDT-testen te houden, het voorschrijven van anti-malaria middelen wordt beïnvloed door de prestaties van de specifieke malaria-RDT die in het studiegebied wordt gebruikt. Zelfs als een malaria-infectie werd gediagnosticeerd met een RDT, was er een neiging om antibiotica voor te schrijven naast anti-malaria middelen. Bovendien is het voorschrijven van antibiotica de standaard geworden wanneer een malaria-infectie werd uitgesloten. Het is duidelijk dat antibiotica worden voorgeschreven uit angst om een bacteriële infectie over het hoofd te zien en zieke kinderen onbehandeld naar huis te sturen, zelfs als een virale infectie de oorzaak van de koorts zou kunnen zijn. De resultaten van malaria-RDT's mogen echter niet van invloed zijn op de beslissing van artsen om elk RDT-negatief kind te behandelen alsof hij/zij een bacteriële infectie heeft.

Artsen hebben dus grote behoefte aan eenvoudige diagnostische richtlijnen om hun klinische beslissingen te sturen. Een klinisch algoritme zou de gemakkelijkste oplossing zijn, omdat hiervoor geen geavanceerde hulpmiddelen nodig zijn. Om de associatie tussen een bacteriële infectie en klinische tekenen/symptomen en basale hematologie te bepalen, werd een univariate analyse uitgevoerd, en variabelen met een p-waarde van <0,1 werden meegenomen in een multivariate analyse (**hoofdstuk 7**). De multivariate logistische regressieanalyse liet zien dat hoge temperatuur (oksel temperatuur>39.5ºC), diarree en oedeem geassocieerd zijn met bacteriële BBIs. Deze associatie is echter niet voldoende specifiek of gevoelig om alle echte bacteriële infecties bij koortsende kinderen jonger dan 5 jaar te voorspellen. In lage- en middeninkomenslanden (LMICs) worden meestal richtlijnen voor het management van koortsepisoden gevolgd. Deze richtlijnen zijn echter slechts oriënterende hulpmiddelen en bieden geen diagnostisch algoritme. Vanwege het lage onderscheidende vermogen van klinische symptomen, zelfs wanneer deze worden gecombineerd met eenvoudige hematologie, is er een dringende behoefte om praktische hulpmiddelen te ontwikkelen om bacteriële infecties te onderscheiden van virale infecties bij kinderen met koorts.

Vanwege de beperkingen van PfHRP2 en pLDH RDTs voor de diagnose van *Plasmodium falciparum*, werden twee diagnostische algoritmen ontwikkeld op basis van sequentiële interpretatie van een twee-lijn malaria-RDT die zowel PfHRP2 als pLDH detecteert (**hoofdstuk 8**). De voorgestelde algoritmen brachten verschillende voordelen aan het licht: (i) malaria werd nauwkeurig gediagnosticeerd bij 84,5% van de kinderen met koorts (diagnostische nauwkeurigheid> 98%); (ii) kinderen met niet eenduidige resultaten, waarvoor malaria microscopie of aanvullende informatie nodig was, werden ook geïdentificeerd (15,5%); (iii) bij kinderen met niet eenduidige resultaten, kan het gebruik van anti-malaria behandelingen in de afgelopen vier weken wijzen op een niet-malaria-infectie; (iv) het gebruik van deze bevindingen zou het onjuist voorschrijven van anti-malaria middelen kunnen verminderen. Het onderzoek suggereert dat een positief of negatief resultaat voor zowel de HRP2- als ook de pLDH-lijn op de RDT nodig is om de diagnostische accuratesse van malaria infecties in endemische gebieden bij 84,5% van de kinderen met koorts te verbeteren. De studie laat zien dat bij afwezigheid van microscopie, de gezondheidswerkers informatie over eerdere malaria behandelingen in de afgelopen vier weken kunnen gebruiken bij kinderen met niet eenduidige resultaten (HRP2 +/pLDH-).

Dit proefschrift illustreert dat de werkelijke oorzaken van koorts al jarenlang worden onderschat door de invloed van malaria op de diagnose van koorts bij jonge kinderen wonend in malaria endemische gebieden. In veel rurale gebieden in SSA is een malaria-RDT het enige beschikbare diagnostische hulpmiddel is om de oorzaak van koorts vast te stellen. Vanwege de beperkingen van deze RDT's om een malaria-infectie correct te diagnosticeren, worden anti-malaria middelen en antibiotica vaak ten onrechte voorgeschreven. Om deze lacune in de diagnose van malaria te overbruggen, kan een twee-lijn malaria-RDT worden geïntroduceerd om zo tot een meer accurate diagnose te komen. Bovendien moet, voortbouwend op de succesvolle implementatie van RDT's voor de diagnose van malaria en het humaan immunodeficiëntievirus (HIV), de introductie van uitgebreide diagnostische kits voor de snelle diagnose van andere veel voorkomende oorzaken van koorts worden overwogen, hetgeen mogelijkheden zou creëren om de behandeling van koorts in rurale gebieden te verbeteren.

## Résume

Au Burkina Faso, la fièvre demeure la première cause de consultation médicale chez les enfants de moins de 5 ans. Pendant de nombreuses années, la fièvre était traitée de façon présomptive. Ce traitement présomptif était étendu aux autres causes probables de fièvre seulement en cas de persistance de la fièvre après un traitement par un antipaludéen. Toutefois, avec l'adoption de la recommandation de l'Organisation Mondiale de Sante (OMS) de diagnostiquer tout cas suspect de paludisme avec un test de diagnostic rapide (TDR) du paludisme, la pratique de diagnostique a changé et cela a conduit à une prescription rationnelle des antipaludéens.

Malgré les efforts à l'échelle mondiale en matière de prévention, de diagnostic et de traitement du paludisme, la fièvre demeure un problème de santé publique majeur dans de nombreuses zones rurales des pays à ressources limitées. L'absence d'outils pratiques pour l'identification des causes de fièvre non-paludéennes conduisent à la prescription systématique des antibiotiques par les agents de sante communautaires lorsque le paludisme est exclu par les TDR. Ce problème devient exacerbant quand les TDR du paludisme ont un niveau de précision diagnostic inferieure à celui attendu, ce qui conduit à des résultats de test faussement positive ou faussement négative. Le but de cette thèse est de mieux comprendre les causes probable (traitable) de fièvre chez les enfants de moins de 5 ans et d'évaluer la valeur ajoutée des TDRs et les pratiques de diagnostic actuelles dans les établissements de soins de santé primaires à Nanoro, au Burkina Faso.

Au **chapitre 2** de la thèse, la littérature sur les étiologies des fièvres non-paludéennes chez les enfants de moins de 5 ans en Afrique subsahariennes (ASS) de 1990 à 2015 a été systématiquement passée en revue. Il ressort de cette revue que le fardeau réel des fièvres non-paludéennes a été sous-estimées durant la période pré-TDR par rapport à la période post-TDR. Parmi les infections non-paludéens investiguées durant ces 25 années, les infections sanguines étaient les plus courantes chez les jeunes enfants, suivis des infections du tractus urinaires et des voies respiratoires. Les étiologies des épisodes fébriles rapportées dans le cas des infections sanguines et urinaires étaient principalement bactériennes. Les étiologies virales rapportées étaient principalement *influenzae* A et B de l'homme et les rotavirus/adénovirus (infection gastro-intestinales). Les infections virales peuvent parfois ne pas être traitée de manière adéquate, mais toutes les autres étiologies (bactérienne ou parasitaire) sont potentiellement traitables et nécessite un diagnostic correct suivi d'un traitement approprié.

Une enquête d'une année sur les causes traitables de fièvre chez les enfants de moins de 5 ans a été conduite dans quatre centres de santé primaire du district sanitaire de Nanoro, ainsi qu'à l'hôpital de référence du district de Nanoro. Les résultats de cette enquête ont été présenté au **chapitre 3**. En plus du paludisme, les infections non-paludéennes, telle que les infections gastro-intestinales, the bactéries commensal potentiellement pathogènes des nasopharynx, les septicémies et les infections du tractus urinaires ont également été investiguées. Parmi les infections non-paludéennes, les infections détectées qui étaient définies comme « la cause probable de la fièvre » dans les cas recrutés étaient les septicémies, les infections gastro-intestinales de type viral et les infections du tractus urinaire. Il ressort de cette enquête que sur 683 enfants, le paludisme reste la première cause de fièvre (49,7%). Des infections non-paludéennes ont été diagnostiquées chez 49,1% des enfants fébriles, parmi lesquelles 10,7%pourraient être « une cause probable de la fièvre ». Trois pourcents (3%) des enfants infectés par le paludisme avaient également une co-infection non-paludéenne pouvant être considérées comme une cause probable de la fièvre. En revanche, chez les enfants non-infectés par le paludisme, 18% des infections non-paludéennes détectées pouvaient être la cause probable de la fièvre. Les autres causes probables de la fièvre étaient significativement associées aux enfants non-infectées par le paludisme par rapport aux enfants infectées par le paludisme ($p<0,001$).

L'étude présenté au **chapitre 4** a montré une différence énorme dans la spécificité des TDRs en fonction de l'opérateur. Les TDRs réalisés par les infirmier(ère)s au niveau des centres de santé primaire ont atteint une spécificité de 52,8%, tandis que ceux réalisés par les techniciens au laboratoire de l'Unité de Recherche Clinique de Nanoro (URCN) ont atteint une spécificité de 74,2%. En effet, la différence entre le nombre de faux positif du paludisme produit par les TDRs réalisés dans les centre de santé et par ceux de la même marque réalisée au laboratoire central de l'URCN était statistiquement significatif ($p=0.0005$). La persistance de l'antigène HRP2 (protéine-2 riche en histidine) après un traitement antipaludéen efficace ne pouvait pas être la cause de la différence observée. Plusieurs facteurs pouvaient expliquer le nombre élevé des cas de faux positifs rapportés avec les TDRs fournis par le Ministère de la Sante y compris probablement des erreurs liées à l'opérateur lors de la réalisation du test et/ou des facteurs biologiques. Bien que peu probables, les conditions de transport et de stockages pouvaient aussi affecter la performance des tests. Les agents de santé doivent être bien formé. De plus, une évaluation périodique sur la réalisation les TDRs du paludisme par les agents de santé et

des contrôles de qualité périodiques des TDRs dans les centres de santé doivent être mis en place.

De manière inattendue, une grande proportion des enfants avec un TDR de paludisme positif étaient des enfants avec une goutte épaisse négative (**Chapitre 5**). Parmi ces enfants, 25% avaient d'autres causes traitables de fièvre (non-paludéennes), qui ne sont pas diagnostiquées si l'on ne tient compte que du résultat du TDR du paludisme à base de HRP2. Parmi les enfants inclus dans cette étude, 49% de ceux chez qui présentaient d'autres causes de fièvre avaient un TDR faussement positif (faux positive). En outre, 36% des autres causes de fièvre étaient diagnostiqués chez les enfants ayant un TDR de paludisme véritablement négatif (vrai négatif) et 15% chez ceux ayant un TDR de paludisme véritablement positif (vrai positive). Sur la base de ces résultats, les agents de santé doivent avoir à l'esprit que si la fièvre persiste après un traitement antipaludéen adéquat, les autres causes de fièvre doivent être prises en compte. Toutes les infections non-diagnostiquées dans le groupe de TDRs faussement positifs étaient traitables et auraient pu causer des maladies graves, voir fatales.

Compte tenu des conclusions du **chapitre 5**, une des conséquences de l'utilisation d'un TDR à base de HRP2 pourrait être la prescription inappropriée des antimicrobiens. Le **chapitre 6** décrit comment, malgré la bonne attitude des agents de santé vis-à-vis des résultats des tests de TDR du paludisme, la prescription des antipaludéens est affectée par la performance du TDR du paludisme utilisé dans la zone d'étude. Même si un TDR diagnostiquait une infection palustre, il y avait une tendance à prescrire des antibiotiques en plus des antipaludéens. De plus, la prescription d'antipaludique était devenue systématique lorsqu'une infection palustre était exclue. Il est évident que les antibiotiques sont prescrits par crainte de ne pas passer à côté d'une infection bactérienne et de renvoyer chez eux des enfants malades non-traités, même si une infection virale pouvait aussi être la cause de la fièvre. Toutefois, les résultats des TDR du paludisme ne devraient pas influencer la décision du médecin de traiter chaque enfant qui présente un TDR négatif, comme s'il avait une infection bactérienne.

Les cliniciens ont donc grandement besoin de directives simples de diagnostic pour guider leur décision clinique. Un algorithme clinique serait la solution la plus facile, vus que cela ne nécessite pas d'outil sophistiquée. Afin de déterminer l'association entre une infection bactérienne et les signes cliniques et symptômes cliniques et l'examen d'hématologie de base, une analyse univariée a été réalisée et seul la variable avec une p-value <0,1 ont été prise en compte dans l'analyse multivarié (**chapitre 7**). L'analyse de la régression logistique multivariée a révélé que les fortes températures (température axillaire >39.5º), la diarrhée et

les œdèmes étaient associés a une septicémie. Cependant, cette association n'est pas suffisamment spécifique ou sensible pour prédire toutes les infections bactériennes réelles chez les enfants fébriles de moins de 5 ans. Dans les pays à revenu faible et intermédiaire, les directives pour le traitement présomptif des épisodes fébriles sont généralement suivies. Cependant, ces directives ne sont que des outils d'orientation et ne fournissent pas d'algorithmes de diagnostic. En raison du faible pouvoir discriminatoire des signes cliniques et des symptômes, même si elles sont combinées avec l'hématologie simple, il est urgent de développer des outils pratiques permettant de distinguer les infections bactériennes des infections virales chez les enfants fébriles.

Compte tenu des limites de *Pf*HRP2 and *p*LDH dans le diagnostic des infections a *Plasmodium falciparum*, deux algorithmes de diagnostic basés sur l'interprétation séquentielles du TDR du paludisme à 2 bandes détectant à la fois *Pf*HRP2 et *p*LDH ont été évalués (**chapitre 8**). Les algorithmes proposés ont révélé plusieurs avantages : (i) le paludisme a été diagnostiqué avec précision chez 84,5% des enfants fébriles (avec une précision>98%) ; (ii) des enfants avec des résultats non concluants, pour lesquels une microscopie du paludisme ou des informations complémentaires étaient nécessaires, ont également été identifiés (15,5%) ; (iii) chez les enfants avec un résultat non-concluant, la preuve de traitements antérieurs d'antipaludéens au cours des 4 dernières semaines pourrait indiquer une infection non-paludéen ; (iv) la prescription inapproprié des antipaludiques pourrait être réduite. En d'autres termes, cette étude suggère qu'un résultat positif ou négatif des 2 bandes HRP2 ou *p*LDH sont requis pour améliorer la précision de diagnostic des infections palustres dans les zones d'endémie palustre chez 84,5% des enfants fébriles. L'étude a suggéré que pour les enfants avec un résultat non concluant (HRP2+/*p*LDH-) et en absence de microscopie du paludisme, les agents de santé pourraient envisager d'utiliser des informations sur les traitements antérieurs d'antipaludéens au cours de 4 dernières semaines. Si des informations sur un traitement antérieur d'antipaludéen peuvent être collectées dans ce groupe, les agents de santé doivent prendre en compte le fait que chez les enfants avec un traitement antérieur d'antipaludéen au cours de 4 dernières semaines, la cause réelle de la fièvre peut ne pas être le paludisme. Par conséquent, les infections non-paludéens doivent être prise en compte.

Cette thèse montre que les causes réelles de la fièvre ont été sous-estimées pendant de nombreuses années en raison de la prédominance du paludisme (dans le diagnostic) chez les enfants. Dans de nombreuses zones rurales d'Afrique Sub-Saharienne, le TDR du paludisme est le seul outil de diagnostic disponible pour établir la cause de la fièvre. En raison des limites

de ces TDRs pour diagnostiquer correctement une infection palustre, les antipaludiques et les antibiotiques sont prescrits de manière inappropriée. Pour combler cette lacune dans le diagnostic du paludisme, un TDR du paludisme a 2 bandes pourrait être introduit pour permettre un diagnostic plus précis. En outre, en se basant sur la mise en œuvre réussie des TDRs pour le diagnostic du paludisme et le virus de l'immunodéficience humain (VIH), l'introduction de kits de diagnostic étendus pour le diagnostic d'autres causes de fièvre devrait être envisagée, ce qui créerait des possibilités d'améliorer la prise en charge des maladies fébriles en milieu rural dans les pays à ressources limitées.

# *Addendum*

## Acknowledgments

This thesis has been made possible thanks to the support of a number of people who have greatly impacted my career and academic training in scientific research. Some have contributed to this thesis in a formal way and others less formally. It is difficult to name each of you individually, but I express my gratitude to you all. I will nevertheless try my best to thank some of you by name.

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I express my gratitude to the members of the PhD examination committee for agreeing to serve on this thesis committee.

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Dear Kiswensida Gwladys Marina Ines, I miss the words to express my gratitude and appreciation for all the efforts you have made in the last 4 years. I was always away when you and the children needed me. But you always found the words to explain to our children why their dad was absent. Thank you for your love, your patience and support during my PhD studies. This thesis is also yours. To Arielle, Anael, Ange and to you, I dedicate this thesis as a recognition of all your support during these last 4 years.

Finally, I am grateful to my parents who have guided my steps since my birth. To my father, I say thank you for life, for the education, for everything. To my mother – gone too early – I

dedicate this thesis in your memory, your good gestures and your good words at the right time. May this work bring you legitimate pride.

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**Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Tropical Medicine and International Health 2016:21 (8):943-955.**

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**Implementation of a malaria rapid diagnostic test in a rural setting of Nanoro, Burkina Faso: from expectation to reality. Malaria Journal 2018: 17: 316.**

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**Accuracy of a *Plasmodium falciparum* specific histidine-rich protein 2 rapid diagnostic test in the context of the presence of non-malaria fevers, prior anti-malarial use and seasonal malaria transmission: Malaria Journal 2017: 16: 294.**

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**Can clinical signs or symptoms combined with basic hematology data be used to predict the presence of bacterial infections in febrile children under - 5 years? BMC Pediatric 2018: 18: 370.**

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Massa dit Achille Bonko, Institut de Recherche en Science de la Santé-Unité de Recherche Clinique de Nanoro, Nanoro, Burkina Faso. Contributed to the study design, data collection and the writing of the manuscript.
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## About the author

Francois Kiemde was born on October 27, 1980 in Abidjan, Ivory Coast (Cote d'Ivoire), where his parents had immigrated from Burkina Faso. He undertook his primary education in Ivory Coast until his father's retirement. Back in Burkina Faso in 1991, he continued his primary and secondary education in the department of Toece (province of Bazega) and Ouagadougou (the capital). He continued his education with studies in chemistry and biology at the University of Ouagadougou, at which he received his Master 1 degree in 2006. He subsequently studied applied biology/biological systems modeling at the University of Bobo-Dioulasso and received his Master 2 degree in 2010.

In July 2009, Francois was recruited by the Clinical Research Unit of Nanoro (Unite de Recherche Clinique de Nanoro, Burkina Faso) as a field supervisor in the framework of the RTS,S-Malaria-055 project (phase III). Following this, he was recruited by the Ministry of Scientific Research and Innovation as a research assistant in June 2014.

In June 2014, he successfully obtained a small grant from the International Society of Infectious Diseases (ISID) to investigate the capacity of *natural killer* cells to lyse erythrocytes infected by *Plasmodium falciparum*, and to determine the factors associated with cytotoxicity. The title of the study was "Determination *in vitro* of cytotoxicity of *natural killer* cells directed against erythrocyte infected by *Plasmodium falciparum* during pregnancy." In October 2014, he was recruited as a PhD student at KIT Biomedical Research, Amsterdam, and subsequently by the Academic Medical Centre in Amsterdam in May 2015. His PhD study was conducted in collaboration with his home institute, IRSS-CRUN (Institut de Recherche en Science de la Santé-Unite de Recherché Clinique de Nanoro). During his PhD research, he worked on the RAPDIF project, which aimed to improve the diagnosis and management of febrile diseases in children under 5 years. His PhD research was financially supported by a grant from the Netherlands Organization for Health Research and Development (ZonMw). Currently, Francois is employed by the Ministry of Higher Education, Scientific Research and Innovation of Burkina Faso, and he is stationed at the Institut de Recherche en Science de la Santé-Clinical Research Unit of Nanaro (IRSS-CRUN, Nanoro).

Francois is married to Kiswendsida Gwladys Marina Ines, with whom they have three children: Arielle, Anael and Ange.

## PhD Portfolio

### Courses

2015

- Clinical Epidemiology: Systematic Review, Amsterdam University Medical Centre, Academic Medical Centre, Amsterdam, The Netherlands.
- Practical Biostatistics, Amsterdam University Medical Centre, Academic Medical Centre, Amsterdam, The Netherlands.
- Infectious Diseases, Amsterdam University Medical Centre, Academic Medical Centre, Amsterdam, The Netherlands.

2017

- Clinical Epidemiology: evaluation of Medical Tests, Amsterdam University Medical Centre, Academic Medical Centre, Amsterdam, The Netherlands.
- Scientific Writing in English for publication, Amsterdam University Medical Centre, Academic Medical Centre, Amsterdam, The Netherlands.

### Specific training

2015

- Norm ISO 15189:2012, Clinical Research Unit of Nanoro, Nanoro, Burkina Faso.

### Conferences and presentations

2017

- *Oral presentation:* **Etiology of fever episodes among children under 5 years in high seasonal malaria transmission area, Burkina Faso. Kiemde F.**, Tahita M. C., Lompo P., Rouamba T., Some A. M., Tinto H., Mens P. F., Schallig H. D. F. H. and Boele van Hensbroek M. 10th European Congress on Tropical Medicine and International Health (ECTMIH), Antwerp, Belgium. 16-20/10/2017.
- *Oral presentation:* **Impact of a malaria RDT-*Pf*HRP2 in the management of other causes of fever in children under 5 years of age in a high seasonal malaria transmission area. Kiemde F.**, Bonko A., Tahita M.C., Lompo P., Boele van Hensbroek M., Tinto H., Mens P. F., and Schallig H. D. F. H. 10th European Congress on Tropical Medicine and International Health (ECTMIH), Antwerp, Belgium. 16-20/10/2017.

2018

- *Oral presentation:* **Implementation of a malaria rapid diagnostic test in a rural setting: from expectation to reality**. **Kiemde F.**, Tahita M. C., Bonko M. A., Mens P.

F., Tinto H., Boele van Hensbroek M. and Schallig H. D. F. H. African Association for Research and Control of Antimicrobial Resistance (AAARAM) 1st Congress, Bamako, Mali 26-28/02/2018.

- *Oral presentation:* **Etiology of fever episodes among children under 5 years in high seasonal malaria transmission area, Burkina Faso**. **Kiemde F.**, Tahita M. C., Lompo P., Rouamba T., Some A. M., Tinto H., Mens P. F., Schallig H. D. F. H. and Boele van Hensbroek M. West Africa Health Research Network (WAHRNET) 3rd Scientific Conference, Cotonou, Benin. 5-7/03/2018.
- *Oral presentation:* **Sequential screening of febrile patients with different malaria rapid diagnostic tests to improve the diagnosis of malaria in the seasonal transmission setting of Nanoro, Burkina Faso**. **Kiemde F.,** Bonko M. A., Tahita M. C., Mens P. F., Tinto H., Schallig H. D. F. H. and Boele van Hensbroek M. Journées des Science de la Santé de Bobo-Dioulasso (JSSB), Bobo-Dioulasso, Burkina Faso, 8-11 May 2018.
- *Oral presentation:* **Whole year performance of malaria rapid diagnostic tests supplied by health ministry for the control of malaria in Burkina Faso**. **Kiemde F.**, Tahita M.C., Bonko M. A, Mens P. F., Tinto H., Boele van Hensbroek M. and Schallig H. D. F. H. Journées des Science de la Santé de Bobo-Dioulasso (JSSB), Bobo-Dioulasso, Burkina Faso, 8-11 May 2018.
- *Poster presentation:* **Implementation of a malaria rapid diagnostic test in a rural setting: from expectation to reality. Kiemde. F**, Tahita M. C., Bonko M. A., Mens P. F., Tinto H., Boele van Hensbroek M. and Schallig H. D. F. H. 19th Journées des Science de la Santé de Bobo-Dioulasso (JSSB), Bobo-Dioulasso, Burkina Faso, 8-11 May 2018.

**Teaching**

2017-2019

- Supervision of master students: one student at the Academic Medical Centre, Amsterdam, The Netherlands and one at the Clinical Research Unit of Nanoro, Nanoro, Burkina Faso.

**Seminar, workshop and master classes**

2015-2016

- Weekly department seminars, Parasitology group, Academic Medical Centre, Amsterdam, The Netherlands.

2018

- Research Method Workshop, Crick African Network, MRC Unit, Banjul, The Gambia.

**List of publications**

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