Acute febrile illness in preschool children in Zanzibar-

Infectious aetiologies, diagnosis and treatment

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”We have not succeeded in answering all our problems. The answers we have found only serve to raise a whole set of new questions. In some ways we feel we are as confused as ever, but we believe we are confused on a higher level and about more important things.”

excerpt from “The Workshop Way of Learning” Professor Earl C Kelley, Wayne University, USA, 1951.

Dedication

This thesis is dedicated to Anita Destinedforgreatness Sackie, R.N, MPH, who sadly died on 1st October, 2014 in Monrovia, Liberia of the disease that she was fighting in her profession. If everyone had a fraction of your bravery, strength and compassion, the world would be a better place. We are forever grateful to you and all health care workers in Liberia for saving us all from Ebola.

ABSTRACT

Background: A majority of the three million children in Africa that do not survive their fifth birthday die from infections that often start as a seemingly uncomplicated febrile illness. Primary health care workers frequently encounter febrile children with a negative malaria rapid diagnostic test (mRDT), in particular in places like Zanzibar with a considerable decline in malaria prevalence. In recent years, accurate and sensitive molecular techniques like the polymerase chain reaction (PCR) have revealed increased detection of pathogens not only in ill patients but also in asymptomatic subjects. These factors underline the importance of re-evaluating the infectious disease aetiology and pathogen dynamics in febrile children and to assess whether existing diagnostic tools like mRDT and fever management guidelines like the IMCI (Integrated Management for childhood illness) remain useful and safe. **Methods and Findings:** The thesis is based on two field studies, both conducted on patients with acute uncomplicated febrile illness (by history or axillary temperature) in primary health care facilities April-July 2010 and 2011 in Zanzibar, Tanzania. In **study 1** (*paper I*), 3890 febrile patients ≥2 months were included. Malaria prevalence by mRDT was 3.1%, with the highest prevalence, 6.1% in children aged 5-14 years old. Malaria microscopy and PCR were conducted on all mRDT positive and a randomly selected 20% of the mRDT negative patients. The sensitivities of mRDT versus malaria microscopy and PCR were below 80%, respectively. **Study 2** (*paper II-IV*) included 677 febrile children aged 2-59 months of age that depending on the clinical picture were subjected to point-of-care tests, PCR analyses (on inclusion and day 14), urine culture and radiological analyses. For comparison, 167 geographically- and age-matched asymptomatic controls from the surrounding communities were recruited for selected PCR analyses. More than one pathogen was detected by PCR in 98% of patients and 93% of healthy controls. After application of study specific diagnostic criteria using clinical characteristics and laboratory results, including a comparison with detection in healthy controls, a cause of fever was assigned to 86%. The most common were respiratory syncytial virus (RSV), influenza A or B, rhinoviruses, enteroviruses, and *S. pyogenes* (Group A Streptococcus) (*paper II and III*). C-reactive Protein (CRP) was the only variable significantly associated with radiological pneumonia. Antibiotics were prescribed to 74% of patients whereas 22% had an infection that required antibiotics (*paper II*). On follow-up after two weeks >80% of the infections were cleared, but almost half of the sampled patients had a new infection on day 14 (*paper IV*). **Conclusion:** The sensitivity of the malaria RDT was relatively low. Thus, more sensitive tools than histidine-rich protein 2 (HRP-2) based mRDTs are warranted. Most of the uncomplicated febrile illness in children in Zanzibar was caused by a viral respiratory tract infection. Comparison of pathogen detection in febrile and healthy children was crucial for identifying cause of disease. The accuracy of the IMCI guidelines to guide antibiotic prescription was suboptimal with both over- as well as underprescription of antibiotics. However, the study did not find any diagnostic tool to help in guiding antibiotic prescription although C-reactive Protein might be a promising biomarker for future intervention studies. Respiratory infections usually cleared within two weeks. However, many children had acquired a new viral infection, suggesting that prolonged symptoms often are due to acquisition of new infections rather than to persistence.

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Zanzibar, en ö utanför Tanzanias kust, har de senaste femton åren haft en unik minskning av förekomsten av malaria. Tidigare var eldriven mikroskopi som gjordes på patientens blod av särskilda laboratorietekniker det enda sättet att veta om en patient hade malaria eller inte. De senaste åren har man utvecklat snabbtest för malaria som gör att hälsoarbetarna inom femton minuter kan ställa malariadiagnos och detta utan att de behöver tillgång till elektricitet.

Trots nedgången i malariaförekonst så dör fortfarande alldeles för många barn under fem års ålder med feber. Vi ville därför undersöka om de nya typerna av malariasnabbtest är säkra att använda. Dessutom ville vi lite noggrannare studera de barn som sökte på vårdcentralen som inte hade malaria genom att använda moderna och känsliga metoder (så kallad PCR). PCR påvisar arvsmassan (DNA eller RNA) från bakterier, virus och andra så kallade mikroorganismer. Vi ville också jämföra förekomsten av dessa mikroorganismer hos febriga barn med barn som mådde helt bra.

Metoder och resultat: Avhandlingen består av studie 1 (genomförd på ett flertal vårdcentraler på Zanzibar och Pemba april-juli 2010) och studie 2 (genomförd på en stor vårdcentral på Zanzibar april-juli 2011). I studie 1 ingick patienter över 2 månaders ålder med feber och alla genomgick ett snabbtest för malaria. Av 3890 patienter var tre procent positiva med malariasnabbtestet. Dubbelt så hög förekomst av malaria (6%) sågs hos barn som var i åldrarna 5 till 14 år. För att veta hur väl malariasnabbtesten fungerar så testades en del av patienterna även med mer känsliga malariatestmetoder (malaria mikroskopi och PCR). Det visade sig att snabbtesten hittade mindre än 80% av de patienter som var positiva för malaria genom såväl mikroskopi som PCR.

I studie 2 ingick 677 barn med feber i åldrarna 2-59 månader. Dessa barn provtogs beroende på vilka symptom de hade, exempelvis blodprov, halsprov och urinprov. På barn med misstanke om lunginflammation (förekomst av hosta och snabb andning) genomfördes en lungröntgen. De känsliga PCR-proverna analyserades på pinnprov tagna från näsa/svalg och ändtarm från både patienter och 167 välmående barn vilka hittades genom att gå från hus till hus i byarna runt omkring vårdcentralen.

Dessutom togs ett nytt PCR-prov för jämförelse på 25% av patienterna två veckor senare.

Hos 86% av patienterna kunde en trolig feberorsak hittas. De vanligaste var luftvägsinfektionsvirus (särskilt RSV och influensavirus) och streptokocker (halsfluss). Men vi såg också att förekomsten av potentiellt sjukdomsorsakande mikroorganismer hos välmående barn var nästan lika hög som hos patienter med feber. Lunginflammation bekräftades med lungröntgen i endast 12% av fallen med klinisk misstanke. CRP (så kallad snabbsänka) var signifikant högre hos dessa barn med röntgenförändringar än de utan. Endast två patienter hade malaria (0,3%) men vi kunde inte hitta några andra tropiska sjukdomar så som denguevirus. Nästan tre fjärdedelar av patienterna fick antibiotika medan endast 22% hade en bakterieinfektion som behövde antibiotika. I samband med uppföljningsprovtagning efter två veckor så hade mer än 80% av infektionerna försvunnit, men hos nästan hälften av patienterna hittades en ny infektion dag 14. Den vanligaste nya infektionen var rhinovirus.

Slutsats: Känsligheten hos snabbtesten för malaria var relativt låg vilket bland annat låg till grund för att snabbtestet byttes ut till ett annat på Zanzibar efter studie 1. Till skillnad från vad man tidigare trott så hade de flesta barn med feber en virusinfektion som försvann inom två veckor, men många fick en ny virusinfektion under samma tid. Det verkar således som om att barn konstant utsätts för nya infektioner men är bra på att snabbt läka dem. Förekomsten av vissa bakterier och virus var i många fall lika vanlig hos patienter som välmående barn. När vi jämförde tillgängliga riktlinjer för antibiotikabehandling såg vi att både över och underbehandling var vanligt.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

Paper I

Shakely D, Elfving K, Aydin-Schmidt B, Msellem MI, Morris U, Omar R, Xu W, Petzold M, Greenhouse B, Baltzell KA, Ali AS, Björkman A and Mårtensson A. *The usefulness of rapid diagnostic tests in the new context of low malaria transmission in Zanzibar*. PLoS One. 2013;8(9):e72912. Epub 2013/09/12.

Paper II

Elfving K, Shakely D, Andersson M, Baltzell K, Ali AS, Bachelard M, Falk KI, Ljung A, Msellem MI, Omar R, Parola P, Xu W, Petzold M, Trollfors B, Björkman A, Lindh M and Mårtensson A. *Acute Uncomplicated Febrile Illness in Children Aged 2-59 months in Zanzibar - Aetiologies, Antibiotic Treatment and Outcome.* PLoS One. 2016;11(1):e0146054.

Paper III

Elfving K, Andersson M, Msellem MI, Welinder-Olsson C, Petzold M, Bjorkman A, Trollfors B, Mårtensson A and Lindh M. *Real-time PCR threshold cycle cutoffs help to identify agents causing acute childhood diarrhea in Zanzibar*. Journal of clinical microbiology. 2014;52(3):916-23. Epub 2014/01/10.

Paper IV

Elfving K, Shakely D, Andersson M, Baltzell K, Msellem MI, Bjorkman A, Mårtensson A, Petzold M, Trollfors B, and Lindh M. *Pathogen Clearance and New Respiratory Tract Infections Among Febrile Children in Zanzibar Investigated With Multitargeting Real-Time Polymerase Chain Reaction on Paired Nasopharyngeal Swab Samples.* Pediatr Infect Dis J. 2018;37(7):643-8.

ABBREVIATIONS

ACT	artemisinin-based combination therapy
AMR	antimicrobial resistance
Arbo	arthropod-borne
ARF	acute rheumatic fever
ARI	acute respiratory tract infection
BCG	Bacillus Calmette Guerin
BCR	balanced accuracy value
BS	blood smear
CBC	complete blood count
CRP	C-reactive protein
CHERG	Child Health Epidemiology Reference Group
CHIKV	chikungunya virus
CHW	community health workers
CI	confidence interval
CMV	cytomegalovirus
Ct	threshold cycle
CXR	chest X-ray
DBS	dried blood spots
DENV	dengue virus
DNA	deoxyribonucleic acid

DPT	diphtheria, pertussis, tetanus
EBV	Epstein-Barr virus
FUO	fever of unknown origin
ELISA	Enzyme Linked Immunosorbent Assays
EPI	Extended Programme of Immunization
EPEC	enteropathogenic *Escherichia coli*
ETAT	emergency assessment and treatment
ETEC	enterotoxigenic *Escherichia coli*
GABRIEL	Global Approach to Biological Research, Infectious diseases and Epidemics in Low-income countries
GAS	group A Streptococci
GBD	Global Burden of Disease
GE	gastroenteritis
GEMS	Global Enteric Multicentre Study
GG1/GG2	geno group 1/geno group 2
HHV-6	Human Herpes Virus type 6
Hib	*Haemophilus influenzae* type B
HRP-2	histidine-rich protein 2
iCCM	Community Case Management system
ICH-GCP	International Conference on Harmonisation-Good Clinical Practice

IHME	Institute of Health Metrics
ILI	influenza-like illness
IMCI	Integrated Management of Childhood Illness
IMCIAB	antibiotic indication by IMCI
InfRA	infection requiring antibiotics
IRS	indoor residual spraying
LLIN	long-lasting insecticide treated nets
LMICs	low- and middle-income countries
LRTI	lower respiratory tract infection
MAL-ED	Malnutrition and Enteric disease
MDA	mass drug administration
MDG	Millennium Development Goals
mRDT	malaria rapid diagnostic test
MTAT	mass testing and treatment
NAAT	nucleic acid amplification test
NMFI	non-malarial febrile illness
NPV	negative predictive value
NTS	non-typhoidal salmonellosis
OPV	oral polio vaccination
OR	odds ratio
PAF	population attributable fraction

PCR	polymerase chain reaction
PCT	procalcitonin
PCV	pneumococcal vaccination
PERCH	Pneumonia Etiology Research for Child Health
PHCC	Primary Health Care Centre
PHCU	Primary Health Care Unit
pLDH	Plasmodium lactate dehydrogenase
POC	point-of-care
qRT-PCR	quantitative real-time reverse transcription PCR
RDT	rapid diagnostic test
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RVFV	Rift Valley fever virus
SARI	Severe Acute Respiratory Tract Infection
SDG	sustainable development goals
SOP	standard operating procedures
SSA	sub-Saharan Africa
WHO	World Health Organization
UNICEF	United Nations Children's Fund
URTI	upper respiratory tract infection
UTI	urinary tract infection

WBC	white blood cell count
WNV	West Nile virus
ZMCP	Zanzibar Malaria Control Programme
ZAMEC	Zanzibar Medical Research Ethical Committee
ZAMEP	Zanzibar Malaria Elimination Programme

CONTENT

INTRODUCTION

Global child mortality has decreased substantially over the past two decades, but still each year almost six million children succumb to easily preventable or treatable diseases[1]. Most of them die in resource limited settings and almost half in sub-Saharan Africa (SSA)[2]. However, they often initially present to primary health care [3] or a drug shop[4] with a seemingly non-severe febrile illness.

Despite the fact that infectious diseases like malaria[5], rotavirus[6], and pneumococcal infections[7] are considered the major killers of preschool children, in-depth studies on aetiologies of uncomplicated childhood febrile illness in Africa are scarce[8]. Epidemiological fever studies often focus on one specific pathogen, diagnosis or disease, or on include severely ill children[9]. Thus, it may be difficult to grasp the cause and proper management of febrile illness[10 11], in contrast to pneumonia and diarrhoea which have been more thoroughly studied[12-16]. Moreover, most fever studies are hospital-based or investigate selected patient groups, some use out-dated techniques, and others do not include healthy controls for comparison [9 17 18].

Zanzibar is an island off the Tanzanian coast that in the recent decades has had an extraordinary success in controlling malaria[19] and is now trying to reach the goal of elimination. This has been achieved through coordinated interventions on vector control, diagnosis and treatment. The island was also one of the first to introduce point-of-care Rapid Diagnostic Tests (RDT) for the diagnosis of malaria. The malaria rapid diagnostic tests (mRDT) were evaluated after introduction and regarded safe and cost-effective[20], but this was in 2005 when every third patient had malaria, whereas in 2010 official figures indicated a tenth of that. Hence it is important to re-evaluate the infectious aetiology and pathogen dynamics in febrile children as well as to assess whether existing diagnostic tools like mRDTs and management guidelines (like the Integrated Management of Childhood Illness- IMCI) including antibiotic treatment remain useful and safe[21].

The thesis highlights aspects of diagnosis, management and treatment of uncomplicated febrile illness in this low malaria endemic setting. It reflects upon which considerations to be made when attributing cause of fever in a preschool child, looking at the patient and the pathogens, risk factors, levels of severity, inclusion criteria and study definitions and also the pitfalls of interpretation as well as clinical management. The project is divided into two studies study 1 (*paper I*) and study 2 (*paper II, III* and *IV*).

The aim of study 1 was to evaluate whether the mRDT, was still an effective tool to identify and rule out *P. falciparum* infection on primary health care level after the recent decrease in malaria prevalence. Would prescribers continue to adhere to the mRDT results when over 95% of the tests were negative and alternative diagnostic tools for fever management were scarce? The aim of study 2 was in addition to decipher aetiologies of non-severe febrile episodes in children by applying sensitive real-time polymerase chain reaction (PCR) techniques also to demonstrate whether the comparison of pathogen load and detection in febrile children versus healthy controls aid in deciding which pathogen that is causing the fever. This final diagnosis was compared with the corresponding IMCI classification and treatment.

Febrile illness has a substantial impact on a low-income society

Fever is one of the most common reasons for parents seeking medical advice for their children in low-income countries[3 22]. The community *fever prevalence* in children, defined as 'history of fever in the preceding two weeks', is estimated to be around between 10-28% in sub-Saharan Africa[23-26] (Shakely et al, unpublished data*), and significantly higher among poorer than wealthier children[25 26]. Also, the younger the patient, the higher is the *fever incidence*[27].

Febrile illness has a substantial impact on the lives of poor people living in malaria endemic countries, and it is also the poorest of the society who have the highest risk of contracting a fever[28]. Still, this is the group of children who has the least access to health care services[29]. The poorer the patient and the more severe the febrile illness, the higher the amount that the caretaker has to pay from out-of-pocket[30 31]. Many febrile illness prevention and treatment strategies, like the World Health Organization (WHO) extended programme of immunization (EPI)[32], malaria control and elimination programs[33] and integrated community management programs[34] are *cost-effective*. However, on a policy maker level, the issue of *affordability* is often just as important. A good example is the management of Acute Rheumatic Fever, (ARF) especially in low- and middle-income countries (LMICs). Primary and secondary prophylactic interventions against group A streptococci (GAS) are regarded as highly cost-effective for the prevention of ARF but the gain is reached after a longer period of time and the public investments before that are quite high and sometimes not affordable[35].

Conclusion: Defining the causes of fever in the poorest young children is particularly important since they have the highest disease burden.

**Unpublished data from cross-sectional studies. Reports of fever in the last 14 days were relatively similar in 2003, 2006, 2007, and 2009 (13%, 12%, 9%, and 15%, respectively).*

Fever definition in low-income malaria endemic settings

Fever is defined in the PubMed Mesh headlines as "An abnormal elevation of body temperature, usually as a result of a pathologic process". Most research on acute febrile illness applies a maximum duration of fever of less than 7 days for included patients. Patients that have had fever for over a week are quite rare in comparison and usually fall in to another category like Fever of unknown origin (FUO) that more often have other aetiologies than infections that explain their condition

like connective tissue disorders, systemic vasculitides and malignancies[36]. Moreover, a distinct study-specific cut-off in temperature that defines a fever is often applied. Examples are case definition of influenza-like illness (ILI) and typhoid fever which both include a measured temperature of >38°C[37 38]. However, enrolling only those patients that are febrile on presentation and excluding those with merely a history of fever could decrease the sensitivity to diagnose some infections. The most evident example is malaria that often manifests with an intermittent fever pattern[39]. Also a thermometer is often not available in the peripheral health centres in SSA. Thus management guideline that includes only a measured fever might be less useful in resource limited settings[40 41].

Consequently, fever studies and clinical algorithms in malaria endemic settings often use a cut-off of 37.5°C in axillary temperature and/or history of fever in the preceding days as a marker for febrile illness. This approach is supported by the fairly high sensitivity of caretakers' report of fever still keeping in mind that fever prevalence may be overestimated[24 42-46]. Thus, parents who deny the presence of fever are often accurate but the opposite must be confirmed. History of fever as a part of febrile illness definition has been an important indicator when performing studies in settings with high malaria endemicity. However, with decreasing malaria prevalence, this notion might be questioned [47], especially in older children[48]. Yet, multiple studies show that no universally applicable criteria exist that differentiate malaria diagnosis from other febrile illnesses in malaria endemic areas. Especially in low endemic areas, malaria microscopy or other parasitological confirmation is required for an accurate diagnosis[48 49].

Conclusion: The definition of fever in malaria endemic low-income countries has to incorporate history of fever to be useful but with decreasing malaria prevalence this could be questioned.

Malaria epidemiology and risk in sub-Saharan Africa

The most important cause of febrile illness historically continuing to this date both considering the impact on morbidity and mortality is the mosquito-borne disease malaria[50]. The most common and dangerous type of malaria is caused by the single-celled, eukaryote protozoan parasite *Plasmodium falciparum*. There are 4 other types of malaria species; *Plasmodium malariae, Plasmodium ovale*, *Plasmodium vivax*, and lastly the most recently discovered *Plasmodium knowlesi*. The first three are all endemic in Africa. *P. knowlesi* and *P. vivax* use the duffy blood group antigen to enter the erythrocyte. In sub-Saharan Africa, the proportion of persons that are so called duffy negative is high[51]. These persons have previously been regarded resistant to these two latter types of malaria parasites[52]. Yet, recently this notion has been questioned, and *P. vivax* may be more common in Africa than previously thought[53 54] but instead illicit low grade parasitaemias in duffy negative persons that previously might have gone under the radar[55].

Global malaria prevalence has declined in the recent decades and also in Africa, in some places more, less in others[19 56]. Still, in some areas half of the febrile patients are positive for malaria[57-59], but even in these circumstances, a great proportion is not explained by *only* malaria[23]. Children and pregnant women are the most vulnerable groups, but everyone affected has a relatively high risk of severe disease or even death, especially for non-immune persons like travellers and migrants moving from a non-endemic to a malaria endemic area. The risk is increased with time from fever onset to diagnosis and treatment, with children typically having a shorter time-span before they reach a severe disease state. The most common and typical symptom of malaria is a fever, whereas severe malaria in children is characterized by signs of shock, hypoglycaemia, severe acidosis and anaemia[60].

Parasitological confirmation of malaria

The current *gold standard* for malaria diagnosis is a standardized microscopy reading of a thick blood smear to define parasitaemia count and a thin smear for species identification[61]. In malaria research studies, two microscopists are used and if there is discordance between the two independent readings, a third and decisive reading will be made. Other traditional laboratory methods like parasite culture might be used for determination of resistance to antimalarial medicines[62]. Immunoassays like Enzyme Linked Immunosorbent Assays (ELISA) tests could be used for mass screening of malaria antigens and antibodies/serology before blood transfusion[63] or as a tool in epidemiological studies on for instance elimination strategies[19 64]. In the recent decades, two new techniques have emerged that have had an impact on malaria epidemiology and clinical management, *malaria rapid diagnostic tests* and *nucleic acid amplification tests* (NAAT)[61] like PCR.

The disadvantages of gold standard malaria microscopy are that it is labour intensive, time consuming, and electricity dependent. The quality of the result also relies on the skills of the microscopist. In routine health care this is often therefore unattainable. Hence, on the first level of health care, malaria diagnosis has historically been made through clinical suspicion without a malaria test. The WHO also used to recommend presumptive antimalarial treatment to all febrile children below five years of age. Malaria RDTs were developed with the purpose to diagnose malaria in peripheral facilities where access to microscopy is unreliable. Initially the tests were relatively expensive and therefore integration of the tests in fever algorithms like the IMCI was regarded not feasible[65]. But gradually the prices were lowered and, consequently in 2010, the WHO changed its global policy to recommend only laboratory parasitological confirmation (by microscopy or RDT) before treatment[66]. This decision was preceded by a debate where those advocating that the mRDT should replace or extend the malaria diagnostic services and be combined with a test-based treatment had the opinion that the previous reasons for a presumptive malaria treatment in Africa were not valid

anymore like high malaria prevalence in febrile children and lack of an effective POC malaria diagnostics, when malaria rapid diagnostic tests were available, accurate[67] [68] and safe[69]. Furthermore, with the introduction of the new and potent antimalarials *artemisinin-based combination therapy (ACT)*, a restricted use only to those patients with parasitologically confirmed malaria infection was regarded necessary. This would avoid development of artemisinin resistance which had been shown in other malaria endemic areas[70] as well as decreasing late diagnosis of malaria negative infections. Researchers arguing for a continued presumptive treatment thought that there was still not enough evidence to support that mRDTs were safe and that ACT prescription could be restricted to only those that were test positive[71].

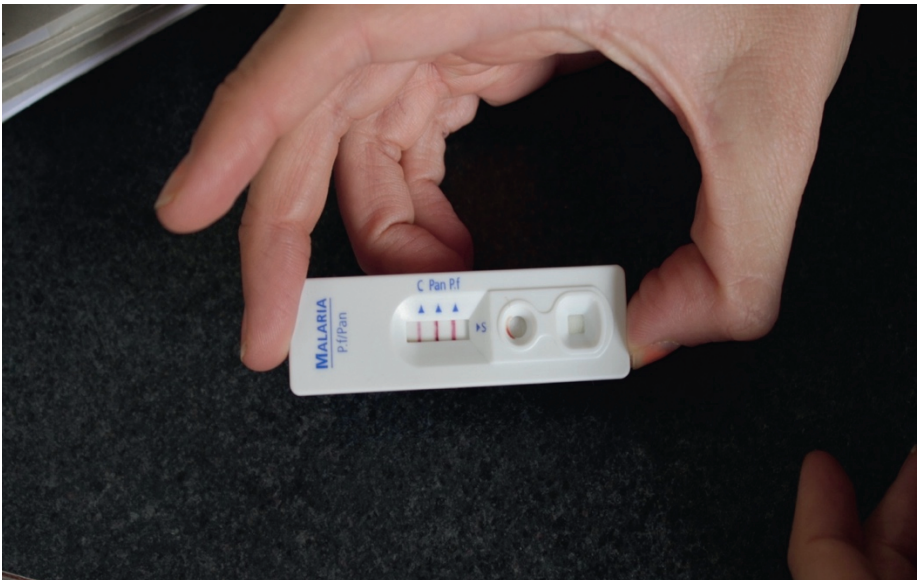


Figure 1. Author of the thesis holding one of the two P. falciparum positive malaria Rapid Diagnostic Tests in study 2. Combination of detection of HRP-2 as well as Pan-specific pLDH. (SD Bioline ®). Photo: Kristina Elfving.

mRDTs are lateral flow immunochromatographic test platforms containing monoclonal or polyclonal antibodies to detect malaria specific antigens in blood via a colorimetric transformation on a nitrocellulose strip (see Figure 1). The technique is similar to point-of-care HIV tests and throat swabs for GAS that give a qualitative test result, i.e. positive or negative with a control line. Reading is easy and could be analysed quickly in 10-20 minutes. Most mRDTs have a detection limit of 200 parasites/uL. There are two basic types of malaria RDTs; *histidine-rich protein 2* (*HRP-2) based tests* that detect only *P. falciparum* malaria and *aldolase* or *Plasmodium lactate dehydrogenase (pLDH) tests* which are both so called pan-specific, i.e. present in all 5 types of malaria species. Thereby distinction between the more fatal falciparum malaria and other malaria species can be made. Figure 1 shows a positive malaria test that combines HRP-2 and pLDH detection for malaria identification. Recently, species-specific mRDTs have been developed also for *P. vivax* [72].

Molecular parasitological confirmation, which is based on more sensitive PCR-techniques, both quantitative and endpoint PCRs, have revealed the presence of low parasitaemias below the threshold of mRDTs and blood microscopy[19 73 74]. NAAT techniques can also help to differ between malaria recrudescence and reinfection[75] as well as to detect molecular markers that are associated with antimalarial drug resistance. This complements the traditional *in vivo* and *in vitro* techniques for measuring malaria parasite resistance[76 61]. Some argue against conventional malaria microscopy. They are in favour of highly sensitive PCR as gold standard for diagnosing malaria in research studies. The argument for is the increased relative importance of patients with parasitaemias below the detection limit of RDT/microscopy as infectious reservoirs, especially in low transmission settings with previous high endemicity and thus high herd immunity in the population[73 74].

Figure 2. Map of Zanzibar. Source: Wikipedia/Oona Räisänen

Zanzibar, a model of success

Zanzibar is comprised of two islands 50 nautical miles from mainland Tanzania (Figure 2) with around 1.3 million inhabitants. This semi-autonomous region has in the last twenty years shown a remarkable success in the control of malaria with a dramatic reduction of *Plasmodium falciparum* prevalence among febrile patients presenting at primary health care facilities[19 77]. This is the result of coordinated, integrated, wide-scale malaria control interventions, which started in 2003 with the deployment of *artemisinin-based combination therapy* (ACT) to all public health facilities.

ACT was followed by vector control measures, initially with the universal distribution of *long-lasting insecticide treated nets* (LLIN) to all pregnant women and children <5 years of age in 2005 and in 2006, universal coverage of *indoor residual spraying* (IRS) of the houses before the rain season was launched. A cornerstone in this comprehensive approach has been parasitological confirmation of the infection instead of presumptive malaria treatment of all febrile patients. Therefore, already in 2006, malaria RDTs were introduced in all public primary health care facilities (so called PHCUs) that were beyond the reach for microscopy and previously had either to refer the febrile patient for malaria microscopy or give presumptive malaria treatment. The first test was a HRP-2 based test[19] (Paracheck ®) which in 2011 was replaced by a combination of analysis of pLDH together with HRP-2 integrated in the same test device (SD Bioline *Pf*/Pan ®, see also Figure 1).

Previously, in Zanzibar, malaria transmission has been stable with seasonal peaks related to the annual rainfall that usually occurs during March to May and October to December. *Anopheles gambiae* complex has been considered the main vector, and *P. falciparum* the predominant malaria species. mRDTs have the potential to improve malaria diagnosis on the first level of care. Concerns have been raised regarding test performance with the risk of high false positivity rates in high endemic settings[78] and false negative rates in low malaria endemic settings like Zanzibar. Hence, sensitivity would be considered the main problem since

low parasitaemias below the threshold of mRDTs might be more clinically relevant[79].

Another issue is the lack of health worker adherence to malaria test results, both positive and negative results, i.e. under and overprescription of antimalarials[80-82]. In 2006 in Zanzibar, HRP-2 based RDT-aided fever diagnosis in primary health care facilities was shown to be efficient for *P. Falciparum* detection, with a sensitivity of 92% and a specificity of 88% when compared with blood smear microscopy. Also, it resulted in improved adequate treatment and health outcome[20]. However, the study was conducted during a time when *P. falciparum* malaria prevalence among feverish primary health care patients was around 30-40%[83]. Now recent research and official figures show that around 2% of patients that present to health care facilities with fever or history of fever have *P. Falciparum* infection and the incidence of symptomatic malaria cases is less than 3 per 1000 inhabitants. The community prevalence of malaria is around 0.1% using mRDT or microscopy whereas the corresponding figure for malaria PCR is 1,8%. Still of note, in 2002, community parasite prevalence by PCR was 25%[19].

Conclusion: There is a need to study the usefulness of mRDT when malaria prevalence has decreased and new molecular techniques have added to the complexity of malaria diagnosis.

Non-malarial febrile illness

With the decrease in malaria prevalence in some areas in Africa [19] there has been an increasing interest in those febrile patients that do not have malaria, especially considering the observed higher mortality rate in febrile children with a negative malaria test in comparison with those who are malaria positive[84]. *Non-malarial febrile illness* (NMFI) was an abbreviation introduced first in peer reviewed literature by Uzochukwu et al in 2009[85]. It has since then often been used as a term for fever episodes in a malaria endemic country that is not caused by malaria, or as defined

by Muro et al; "the situation of a patient with suspected malaria and a negative parasitological test"[86]. Research on NMFI has often been focused on diagnosing invasive bacterial infections and not so much on viral infections or uncomplicated fevers. The dichotomous variable in the NMFI definition; fever with or without malaria has been questioned to be too binary, since co-infections with malaria and other pathogens, like non-typhoidal salmonellosis are quite common[10 87].

To exemplify this, a study from Tanzania on children 6 months to 15 years of age with different levels of disease severity, conducted in two outpatient departments; one hyper endemic (46% of patients had malaria) and one with lower endemicity, showed bacteraemia in around 3% of patients. Interestingly, bacteraemia prevalence was similar in the NMFI patients as in the malaria patients and overtreatment with both antimalarials as well as antibiotics was common[88]. In the hyperendemic site overtreatment with antimalarials to malaria negative patients was associated with more than one IMCI danger sign, prolonged fever and high fever[89]. This may not be so surprising considering the notion that most patients do have malaria and the perceived high risk of missing a severe malaria case might play an important role.

Yet, over diagnosis of malaria in severely ill children could be dangerous since it could mislead the clinician from diagnosing the actual cause of the severe disease[90] Thus, many have advocated simultaneous administration of intravenous antibiotics in these children[91]. But how should the clinician manage this issue in the less sick children? The problem of symptom overlap in febrile children between malaria and pneumonia, like vomiting and rapid breathing has been well described[92 93] but, in addition, these symptoms could also be cardinal symptoms for acidosis, pain and congenital heart and lung disease.

Conclusion: There is a need to study the causes and the management of non-malarial febrile illness especially in areas like Zanzibar that have had a decline in malaria prevalence.

Attributing cause of fever using clinical case definitions

Accurate *clinical case definitions* are useful for research and public health disease surveillance, not only to diagnose infections causing febrile disease in a patient but also to serve as a tool in epidemiologic surveillance like outbreak alerts and evaluation of disease control interventions.

The ideal case definition needs to be concise, uncomplicated, useable, and based on a combination of signs and symptoms that uniquely identify the disease of interest[94]. However, case definitions using merely clinical signs and symptoms are seldom both sensitive and specific. Instead, one has to make a trade-off depending on whether it is more important to identify or to rule out the infection[48], both when studying the epidemiology and seasonal patterns of a pathogen and when managing the patient. For example, in a measles outbreak, finding the patients who are transmitting the disease is more important and false positive cases might not have such great consequences as false negative cases. Conversely, a high rate of false positive tuberculosis diagnoses could be more troublesome due to the long and often complicated treatment.

Hence, clinical case definitions are often not optimal as the only tool. This is especially true in infants and when unspecific disease manifestations are typical of a certain disease or if there is significant symptom overlap between common diagnoses like in arbo- (arthropod-borne) viral infections[95 96], typhoid disease/enteric fever[97 98], pneumonia[92 93] influenza[27 18] and malaria[99]. For dengue virus this has become even more evident in the recent decade with newly discovered or re-emerging diseases like chikungunya and zika virus that all have overlapping symptoms of fever, rashes and arthralgia/myalgia. Initiatives have been made to increase the utility of case definitions by adjusting the criteria in fever epidemiology studies. Here are two examples; in an epidemiological situation where all three arboviruses zika, dengue and chikungunya co-exist, the absence of fever in a patient made the zika diagnosis more

likely[100]. Adding the measured fever to the WHO pneumonia definition increased specificity to diagnose radiological pneumonia from 16% to 50%[101].

Despite the above mentioned difficulties, a large study on febrile disease in Tanzania[102] showed that it was actually possible to decipher some clinical signs or symptoms that point towards a specific aetiology of fever. They found that the strongest predictor for *ruling in* malaria was a high body temperature of >40°C, for typhoid; abdominal tenderness, for radiological pneumonia; abnormal chest auscultation and for any type of bacterial disease; chest indrawing. The strongest predictor to *rule out* urinary tract infection (UTI) was patient age ≥3 years.

Thus, planning of clear entry points and structured case record forms in a research study on febrile illness aetiology are vital and could include presence of *concomitant symptoms or signs* like convulsions, watery or bloody diarrhoea, clinical pneumonia or rapid breathing. One example is the different clinical case definitions of influenza.

Influenza-like-illness (ILI) was initially defined as a fever with a cough and/or sore throat[27]. *Severity of illness* is part of another similar influenza case definition, SARI (Severe Acute Respiratory Tract Infection)[103 104]. Yet, Jones et al showed that influenza detection was higher especially in older children if instead of SARI, the syndrome "acute febrile illness" was used which in contrast to both ILI and SARI does not contain any respiratory component[103]. In line with this, in a cross-sectional influenza study from Senegal on febrile persons found in their home, between 7-18% of febrile participants were positive for influenza of whom only half actually qualified for the ILI syndrome[27]. This was confirmed by studies from Kenya and Singapore where the ILI syndrome was suboptimal to diagnose laboratory confirmed influenza whereas the syndrome "cough + fever" without the criterion sore throat showed a good sensitivity and specificity[37 105].

Consequently, in 2011, in the aftermath of the 2009 H1N1 pandemic, WHO made revisions of both the ILI and SARI syndromes with the goal

to increase sensitivity without compromising specificity to detect influenza infection. The major change was to omit the sore throat criterion from the definition and subsequently influenza-like-illness was defined: "An acute respiratory illness with a measured temperature of ≥ 38 °C and cough, with onset within the past 10 days"[94].

Similar to influenza disease, there is a need for a consensus case definition for respiratory syncytial virus (RSV) studies in low-income countries. Rha, Dahl et al showed that the SARI syndrome only detected 51% of RSV cases <3 months of age and by removing the fever criterion from the SARI and use that as a case definition for RSV, the detection increased to 98%[106]. The authors also pointed out that fever was more common in children >12 months of age in RSV positive versus RSV negative children with SARI. Thus, the study highlights the importance of including *age-specific criteria* in a clinical case definition.

Case definitions of diarrhoeal disease are usually better defined and thus more easily comparable between research studies and report systems. WHO regards diarrhoeal disease as "passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual". However, most studies on diarrhoeal aetiology use the more precise definition of ≥3 loose stools within the previous 24 hours and also include a maximum duration of symptoms to 7 days[107 12] to define an acute diarrhoeal episode. The quality of the diarrhoea is evaluated to further characterize the population, i.e. *watery or bloody diarrhoea* which indicates a mucosal inflammation, and *dehydration status* which indicates disease severity[108]. Hereby, the case definitions are also useful in an acute diarrhoeal outbreak situation of for example shigellosis or cholera.

Narrowing or broadening the criteria for clinical case definitions must be done with caution, especially if used in clinical practice. The decision of a primary health care worker to treat an illness based on only caretaker history and clinical signs has been shown to save lives in low resource settings both for malaria[109] and pneumonia[110], two major child killers in Africa.

Conclusion: Previous reports on causes of fever have not always been comparable due to differences in the studied parameters like type of pathogens, different levels of severity and age categories. Hence, it is vital to standardize case definitions and inclusion criteria both in public health surveillance and as well as aetiology studies, to make the results reproducible and translatable to other settings.

Clinical algorithms for febrile children low resource settings and change in epidemiology

With a decline in malaria incidence, the health worker increasingly encounters a febrile patient with a negative mRDT. The fact that places like Zanzibar despite the lower malaria prevalence continue to have a high child mortality rate underlines the importance of providing evidence on other aetiologies of fever than malaria in children. In this new epidemiological context it is important to re-evaluate the aetiology of acute uncomplicated childhood fever and also to assess whether existing diagnostic guidelines and management tools remain useful and safe[21], especially considering identification of infections that would require antibiotics.

The *Integrated Management of Childhood Illness* (IMCI) guidelines[111 112] were developed by the WHO and the UNICEF to improve primary health care workers' clinical management of preschool children focusing on the main conditions contributing to under-five morbidity and mortality. The goal was to move away from vertically oriented health programmes and to integrate knowledge into one holistic approach, which would be patient-centred rather than disease-oriented. IMCI is comprised of simple stepwise algorithms based on specific entry symptoms and clinical signs that are found on clinical assessment like fever, ear pain, diarrhoea and respiratory rate. These parameters correspond to a classification and a recommended treatment and management including referral for severe patients. In addition, it has an integrated preventive focus on vaccines,

nutrition and anaemia (including antihelmintics) as well as recommendations for follow-up depending on disease classification at initial visit. Moreover, the IMCI has since its start two decades ago a comprehensive family/community approach and focus on improvement of case management skills of health care workers and strengthening of the health system where they work[21 113 114]. The IMCI was also used as the foundation when developing the Community Case Management system (iCCM) used by community health workers (CHW) to manage common childhood illnesses including the fever syndrome in the community[115].

The emergency assessment and treatment (ETAT)- strategy was developed to be used complimentary to the IMCI or iCCM for children needing prompt emergency care and referral management[116]. Despite the fact that these three guidelines are designed to be used in the same settings, there is sometimes confusion as to which guideline to use and where and how to implement or scale it up[117]. Subsequently, results from studies evaluating one of the guidelines can not immediately be generalised to all three. For example, CHWs are able to manage malaria and pneumonia effectively and safe in their community through the iCCM strategy[118] and mRDTs probably reduce the overtreatment with antimalarials but might increase antibiotic use instead[119]. However, this does not necessarily mean that the results are translatable to the health facility based IMCI or ETAT, although the guidelines seem similar in their structure.

Despite the fact that the IMCI is the guideline recommended for children who reside in LMICs, the majority of studies have up to now focused mainly on IMCI implementation, supervision[120], and health worker adherence to the guideline[121 122]. After the launch of the guidelines in the end of the 1990s, there were some studies from India that reported clinical utility of the integrated approach of the guideline. They also showed superiority of the IMCI in comparison with the vertical approach guidelines, in particular when the caretaker had multiple complaints[123-125]. But after that, only a few guidelines focused on the usefulness and safety of the clinical management recommendations or suggestions for further

guideline adaptation for conditions like pharyngitis and severe pneumonia[126-130]. A recent Cochrane report[129] shows that when IMCI management does reach the child, it is cost-effective[131] and increases quality of care[128 129 132], e.g. it improves pneumonia case management[110], rational use of antimicrobials[133 134] and antimalarials. However, IMCI was developed when global child mortality was almost double of what it is today. Thereafter, there have been changes in infectious disease epidemiology of childhood febrile illness following e.g. introduction of new diagnostics like malaria RDT and preventive measures like up-scaled malaria interventions and immunizations against pathogens like rotavirus[135] and pneumococci[136]. Hence, concerns have been raised that the IMCI technical base is old[113], not evidence based[21] and most importantly seldom actually adjusted for local epidemiology[137].

Conclusion: It is necessary to re-evaluate IMCI in new epidemiological and diagnostic contexts, especially when malaria prevalence is declining and new rapid tests are being implemented[121]. This is particularly true in Zanzibar, which already in 2009 was one of the first areas to include the mRDT in a locally adapted IMCI version of the IMCI guidelines[111 138].

Pneumonia diagnosis in resource limited settings

Pneumonia is according to global epidemiology studies the largest killer in children. The annual global death toll is more than 700 000 children under 5 years of age, half of which are thought to occur in Africa, although estimates are relatively uncertain[7]. Paediatric pneumonias manifest in a majority of the cases with a fever[139]. However, there is no existing gold standard for pneumonia diagnosis neither in high-income[140] [141] nor in low- and middle-income settings. Hence, previous pneumonia-focused literature, guidelines or research are rather heterogenic, especially for preschool children[140] [142] [143].

IMCI defines a pneumonia that requires antibiotics as the 'presence of cough and/or difficult breathing in a child with rapid breathing' (age-dependent cut-offs to define increased respiratory rate). The child should be evaluated for tachypnoea when it is afebrile, calm and not hungry, which sometimes might be difficult to attain especially in a low-income setting[144]. The pneumonia algorithm within the IMCI guideline is similar to the previous lower respiratory tract infections (LRTI) guideline launched by the WHO. The development of this pneumonia strategy in the 1980s was based on studies performed on children with pneumonia diagnosis in the US[145], which showed that the presence of fast breathing had a good sensitivity to rule in pneumonia.

The results were confirmed in studies in low-income countries including sub-Saharan Africa[139] [146] and consequently, tachypnoea became central in the pneumonia diagnosis strategy. But already these studies, which formed the basis for the LRTI strategy identified the same problems that afterwards have been shown in follow-up studies such as inability of health care workers to identify danger signs and the importance of training and supervision. Also, recently after the introduction of pneumococcal vaccine studies conducted in paediatric emergency rooms in the US, showed on the contrary a low sensitivity (34,3%) of the WHO pneumonia cut-offs to rule in pneumonia[147].

Due to the lack of a gold standard, even in high-income settings, the decision of a qualified physician to treat the child for a bacterial pneumonia is often used to define a pneumonia diagnosis. This lack of a clear-cut objective endpoint for pneumonia diagnosis often impedes scientific and programmatic evaluation of pneumonia and LRTI management strategies.

The closest to a gold standard is a pneumonia identified on a chest X-ray (CXR). CXR is recommended by the WHO to define the endpoint *radiologically confirmed pneumonia* in most pneumococcal vaccine effectiveness studies and there is also a systematic guideline on interpretation[148]. The CXR interpretation is divided into four groups "endpoint consolidation" which should represent a radiologically verified pneumonia, "other infiltrate" which should not be regarded as pneumonia and "normal" and "uninterpretable". There are also standardized instructions on how to digitalize analogue pictures for interpretation and reading to be performed elsewhere than on-site. Similar to studies on malaria microscopy, reading should be performed by two radiologists and if there is disagreement a third and decisive reading should be initiated.

Conclusion: Radiologically verified pneumonia is still the best at hand gold standard instrument in pneumonia research.

Antibiotic resistance

Many children all over the world do not have access to a correct antibiotic treatment when they need it due to lack of money, a high cost of the most efficacious antibiotics, inappropriate use, and counterfeit drugs[149-152]. Another change in infectious disease epidemiology in the recent decades are the increasing rates of antimicrobial resistance (AMR), which is not only affecting high-income countries but also SSA. WHO has defined antibiotic resistance as a major threat to global health.

AMR should be tackled on the following fronts: Public and health worker awareness, optimized use of antibiotics in agriculture and human health, strengthened research and surveillance, investment in human capital, increased infection control (sanitation, hygiene and prevention), increased investment in new therapeutic approaches (including antibiotics and vaccines), diagnostics tools and other interventions[150].

It is suggested that the problem of antibiotic resistance might even be higher in LMICs than high-income settings[152-154]. Despite this, few efforts have been put in place to tackle the problem of resistance in low-income settings also considering that the lack of expertise is probably even higher there. Naturally, the global IMCI strategy could be suitable as a focal point in the work against AMR. One particularly important area of interest within the IMCI would be the pneumonia/LRTI algorithm due to the high rate of overprescription of antibiotics. Furthermore, there is a worry that following the decline in malaria prevalence, febrile children who previously would have received presumptive malaria treatment through clinical recommendations would now receive antibiotics for a suspected pneumonia instead[155]. This is true in particular when considering the great symptom overlap between malaria and pneumonia described above.

Conclusion: Changes in causes of febrile disease in sub-Saharan Africa, decline of malaria prevalence in some areas and increased antibiotic resistance call for re-evaluation of the antibiotic recommendations in guidelines like the IMCI.

Invasive bacterial infections in febrile paediatric outpatients in Africa

It is difficult to draw any conclusion on the burden of invasive disease in children with uncomplicated febrile severe illness. Fever studies in sub-Saharan Africa often include a mixture of patients with different levels of severity, have a wide age span (from neonates to old adults), and include children with measured fever only. The risk of contamination of blood cultures with skin bacteria from the patients makes the interpretation more difficult[156]. Typically, bacteraemia frequency in these heterogenic patient groups is less than 10% and the most common pathogens isolated are *S. pneumoniae, S. typhii,* non-typhoidal salmonellosis (NTS)*, E.coli,* and *S. aureus*[157-163].

The few studies from SSA report a bacteraemia prevalence of around 1-1.5% in children with non-severe illness[68 164 165]. The most common pathogens detected are e.g. pneumococci and invasive salmonellosis, similar to the community-acquired bacteraemias in more severely ill children[68 164]. Yet, most of the studies have been performed in a setting before the introduction of pneumococcal vaccines in the routine vaccination programme, the EPI. African children are to an increasing extent receiving pneumococcal vaccination (PCV). In addition to an increase of invasive non-PCV pneumococcal types after the PCV introduction, (so called serotype replacement) which has been reported in most places that introduced the vaccine[166], one could also predict that typhoid and other gram negative bacteria might increase their relative part of invasive disease, in preschool children.

Invasive NTS are common in infants living in SSA and have a documented association with malaria infections[167 168], whereas most previous studies report a significantly higher typhoid incidence among older children. Still, *S. typhii* is a well-documented bacteraemia pathogen also in children under 5 years of age[68 98 157 161] and in areas with decreasing malaria incidence, typhoid takes a greater part of the invasive salmonellosis[87]. This possible rise of gram-negative bacteria taking a

greater toll of the IBI as well as a possible increase in AMR could have implications on guidelines for empiric antibiotic use in children with suspected severe febrile illness.

Conclusion: One percent of preschool children with febrile disease without danger signs have bacteraemia of which most are *S. pneumoniae, S. typhii, NTS, E. coli,* and *S. aureus.*

Attributing cause of fever using molecular techniques

Molecular methods developed in the recent decades, have led to an improved understanding of infectious disease epidemiology[14 15]. Conventional methods used before molecular techniques were put into practice like serology, bacterial and viral culture or other types of antigen detection are often time-consuming with mediocre levels of sensitivity and specificity[169-173]. The most common type of molecular technique or nucleic acid amplification technique (NAAT) is the *Polymerase Chain Reaction (PCR)* which can detect genetic material from all kinds of species. It has revolutionised the understanding of in particular infectious diseases with its increased sensitivity to detect pathogens from a sample[174 175].

The pathogen concentration can be measured by the real-time PCR technique which gives the *Ct (threshold cycle) value,* a parameter that is negatively proportional to the logarithm of the target concentration. The real-time PCR also allows for simultaneous detection of multiple agents. Moreover, sensitive multi-targeting techniques have revealed that asymptomatic individuals frequently carry pathogens, not seldom to the same extent as patients, with both conventional[176 177 15], and molecular methods[15 178-181]. Also, there has not only been an increased crude pathogen detection but also higher detection rates of multiple infections in the same individual, so called, *co-infections*[182]. Despite this, many studies

using these molecular methods still do not include *healthy controls* as reference for their interpretation of for example fever aetiology.

Recently, these sensitive techniques have been applied in research in LMICs as well[14 15 18 158] and a more complex picture has emerged. In general, a high detection frequency has been observed also among healthy controls[179], comparable to frequencies in symptomatic respiratory and gastrointestinal infections. In addition, discoveries of new pathogens have been made, in particular viruses like metapneumoviruses, bocavirus and new types of coronaviruses, enterovirus and rhinovirus[183]. However, especially in low-income settings, there is not enough evidence to answer the question, whether the high detection rates and frequent finding of co-infections are due to frequent exposure to new pathogens or if the same pathogens persist for a longer period of time after the actual symptoms have disappeared. Longitudinal sampling after initial detection could help to answer this question.

Despite the lack of a standardised quantification, real-time PCR threshold cycle (Ct) values are acceptable estimates of target concentration, for example, to distinguish which samples to choose for further molecular analyses like sequencing[184] or to get a rough appreciation of level of malaria parasitaemia[185]. But in the recent decade Ct values have also gained interest for their usefulness in evaluation of symptomatic or asymptomatic infections, prediction of disease severity or length of hospitalization. The possible utility has been shown both for diarrhoeal pathogens[186] like norovirus[187], rotavirus[188 189], and *Shigella* infections[190] as well as respiratory tract pathogens like bocavirus, *Bordetella pertussis*, RSV, human rhinovirus, *Haemophilus influenzae*, and *Streptococcus pneumonia*[163 181 191-200].

Conclusion: Smaller reports have been published on real-time PCR detection of viruses and the clearance of for example for RSV and influenza viruses, but few analyse virus detection simultaneously and longitudinally. It is important to include healthy controls for test result interpretation of sensitive molecular techniques.

Global estimates on child mortality and morbidity

Global estimates on child mortality including LMICs are produced by two large groups, the Institute of Health Metrics IHME that produces the annual reports on Global Burden of disease in all age groups (http://www.healthdata.org/) and the WHO/UNICEF associated Child Health Epidemiology Reference Group (CHERG) (www.cherg.org). Both groups use a combination of vital registration and verbal autopsy data. Where official data is incomplete or even non-existent, statistical assumptions and calculations based on data from nearby estimates are made. Thus, these two groups use the same types of data but have different ways of reaching the mortality estimates why the published numbers differ sometimes quite a lot[201].

One commonly used calculation in these types of studies is the *population attributable fraction (PAF)* when assessing the aetiology of a certain disease or symptom. PAF defines the contribution of each pathogen to the overall load of illness[14 180 202] and it can be regarded as the proportional reduction in morbidity or mortality if the exposure to the pathogen was zero. Hence, these studies estimate the burden of disease on population level but do not define a final diagnosis for each individual patient[203]. Hereby, the clinical utility of the information might be limited whereas the information is more useful when deciding on an introduction of a preventive public health intervention e.g. which vaccine to introduce.

RSV could serve as an example. RSV has been regarded the second most common cause of death globally by the Global Burden of Disease (GBD) study, accounting for 27% of deaths in children under 5[7]. However, it might be difficult to say that the cause of death actually is the RSV *per se*, i.e. did the child die with RSV rather than from the RSV, or was it a co-infection or rather a co-morbidity that caused it. A recent retrospective analysis of 358 RSV deaths in research studies from different parts of the

world (RSV GOLD) shows that around half of the children (30% in LMICs and 70% in high-income countries) that die have a comorbidity, of which the most common is congenital heart disease, which is important to recognize if launching an RSV vaccine[204]. Looking only at the RSV contribution to deaths may miss the target.

Conclusion: It is crucial to conduct clinical trials that provide regional or local data on fever epidemiology, especially in LMICs. Registry data and extrapolated figures from studies like the GBD is not enough to understand the syndrome of fever, its causes and outcome, in particular in young children.

AIMS AND OBJECTIVES

The aim of the thesis was to assess the different components of fever diagnosis, management and antibiotic treatment in children below five years of age in Zanzibar with a specific focus on interpreting fever aetiology based on pathogen detection. The thesis is based on two large studies on febrile illness in children, study 1 (*paper I*) and study 2 (*paper II, III and IV*)

Main objective study 1

To determine the effectiveness of malaria RDT used on the first-level health care in a low-malaria endemic setting

Secondary objectives study 1

- To determine accuracy of malaria RDT versus microscopy reading of blood smears (BS) as well as versus parasite detection by PCR.
- To determine the malaria prevalence among fever patients presenting at primary health care centres (PHCCs) and primary health care units (PHCUs).
- To determine health worker adherence to RDT results, i.e. frequency of prescription of antimalarial drugs in RDT positive and negative patients, respectively

Main objective study 2

To determine the causes of uncomplicated fever in children aged 2-59 months presenting to an outpatient department in Zanzibar.

Secondary objectives study 2

- To compare the proportions and quantitative concentrations of certain viral and bacterial pathogens in faeces and nasopharynx in patients and a control group consisting of asymptomatic, age and sampling-time period matched children. (*paper II and III*)
- To evaluate the potential utility of threshold cycle (Ct) values, to separate symptomatic from asymptomatic infections.
- To describe health outcome in patients by follow-up day 14. (*paper II, IV*)
- To compare clinical IMCI classifications and corresponding antibiotic treatment at enrolment according to the new IMCI guideline with a final retrospective diagnosis (derived from collected clinical, laboratory and radiology data) and its corresponding antibiotic treatment (*paper II*)
- To compare levels of C-reactive Protein (CRP) in IMCI pneumonia patients with and without out X-ray verified pneumonia. (*paper II*)
- To analyse the short-term longitudinal dynamics of pathogens including clearance, shedding and new infections detected in the nasopharynx by using multiplex real-time PCR. (*paper II, IV*)

MATERIALS AND METHODS

The laboratory, statistical and epidemiological methods are described in detail and referenced in the published articles that are included in the thesis (see list of manuscripts above, *paper I-IV*).

Figure 3. Study sites and shehia distribution in Micheweni (top right) and North A (lower right) districts, Zanzibar

Study sites and training

Study 1 and 2 were health facility based observational studies conducted in the rural districts of North A in Unguja Island and Micheweni in Pemba Island in Zanzibar (Figure 3).

Each district has around 100 000 inhabitants. In each district there is one Primary Health Care Centre (PHCC) which includes an inpatient department and laboratory services, e.g. blood transfusion and malaria microscopy services. Basic medical treatment services, including malaria RDT aided diagnosis of fever patients, are provided in altogether 12 Primary Health Care Units (PHCUs) per district. If referral is needed, the patients are sent to secondary or tertiary level hospitals. Registered nurses, with 3 to 4 years of formal training, are responsible for providing outpatient care in the PHCUs and clinical officers or assistant medical officers in the PHCCs.

All study personnel were previously trained in malaria case management (diagnosis and treatment and the use and interpretation of mRDT) from the Zanzibar Malaria Control Programme (ZMCP) (which changed name to ZAMEP- Zanzibar Malaria Elimination Programme in 2013) and District Health Management Teams of the Zanzibar Ministry of Health and Social Welfare.

In both study 1 and study 2, the participating study health workers had training on study-specific procedures as well as the recently adopted IMCI guidelines in Zanzibar into which mRDT had been integrated (the latter in preparation for study 1 conducted in 2010). All laboratory investigations but the mRDT that were performed in study 2 were not part of routine IMCI clinical management in Zanzibar but had to be specifically introduced, trained and implemented for the study. Prior to both studies a pilot study with "dummy runs" were conducted.

The under-five mortality rate in Zanzibar was 56/1000 live births according to the 2015/2016 country wide interview survey Tanzania Demographic Health survey- TDHS. This is a clear decrease in

comparison to previous measurements with an under 5 mortality rate of 73/1000 in 2010, 101/1000 in 2004 and 108/1000 in 1996. The decrease is like in many places probably not only due to successful malaria interventions[19] but also introduction of new vaccines as well as increased uptake of already existing immunizations and an increased focus on maternal and neonatal care.

Zanzibar and Tanzania follow the WHO recommended immunization scheme. During the field studies, EPI immunization programmes against diphtheria, pertussis, tetanus (DTP), polio (oral polio vaccination- OPV), tuberculosis (Bacillus Calmette Guerin- BCG), hepatitis B, and *Haemophilus influenzae* type B were in place. The estimated coverage of three dose of DPT was 91%. The most recent vaccine introductions were hepatitis B in 2002 and within the pentavalent DPT-Hepatitis B-Hib vaccine formulation, *Haemophilus influenzae* type B (Hib) immunization was introduced in 2009. In 2013, after completion of the present study, introduction of the 13 valent pneumococcal vaccine (PCV13) and rotavirus vaccine (Rotarix®) were made[135] and in 2014, a second dose for measles was introduced.

Laboratory training, supervision and logistics

Laboratory specimens and reagents were sampled, stored and conducted according to the manufacturers' instructions including quality control measures. Around 100 study specific Standard Operating Procedures (SOP) covering all aspects of the study, including collection and storage of specimens were produced and used as reference for staff at the study site. Laboratory analyses of the samples were performed on different levels; on the study inclusion site, in the Zanzibar microbiology department (Mnazi Mmoja Hospital), in Sweden (the University of Gothenburg or the Karolinska Institutet), and in France (Aix Marseille Université).

All study staff handling laboratory samples were trained on a safe and proper sample collection and were further supervised by the designated study coordinator. After field trial completion, all samples collected for molecular analysis were transported in a controlled environment to Sweden by air, either as dried blood spots (DBS) for blood PCR or on dry ice (for rectal and nasopharyngeal swabs) or in closed preservation agar (for urine cultures). The results from the molecular analyses were available months after patient inclusion was finished and thus did not affect patient management.

Ethical considerations

All studies were conducted in accordance with the principles stated in the Declaration of Helsinki and International Conference on Harmonisation-Good Clinical Practice (ICH-GCP). Before study start, all study protocols, case record forms and consent forms were approved by the Zanzibar Medical Research Ethical Committee (ZAMREC) and the Regional Ethics Committee in Gothenburg and Stockholm for laboratory analyses in the Karolinska Institute and the University of Gothenburg. All patients or their legal guardians presented a written (proxy) consent before study inclusion. All study investigations and medications were provided free of charge. Both study 1 and 2 were registered on clinicaltrials.gov.

Specific methods study 1

Patients aged ≥2 months with a measured fever (electronic axillary temperature ≥37·5°C) and/or a history of fever in the preceding 24 hours were recruited from May to July 2010 by 33 nurses and clinical officers with prescription rights from 12 health facilities (6 in the North A district and 6 in the Micheweni district). Study exclusion criteria were signs of severe disease (as defined by IMCI for children<5 years of age) and previous study enrolment in the last 28 days.
The health facilities were selected for an even geographical distribution and based on the skills of health workers in the PHCU. Also, in each

study district, one PHCC was included where also mRDTs were introduced for the purpose of the study, despite the fact that PHCCs and hospitals in Zanzibar use blood microscopy as the routine malaria diagnostic tool. All patients below 5 years of age were managed by the IMCI guideline and data was documented in a structured IMCI-based case record form.

Malaria analyses

All patients were subjected to an mRDT, performed and interpreted according to the manufacturer's instructions. The mRDT used in the study and deployed by the ZMCP was the Paracheck® detecting *P. falciparum* specific HRP-2 antigen. For those patients who were mRDT positive, additional confirmatory analyses with blood microscopy (thick smear) and malaria PCR on DBS (filter paper Whatmann ® 3MM) were performed. The same confirmatory tests were conducted in 20% of randomly selected mRDT negative patients. Blood smears were prepared on site, and examined by two independent and experienced microscopists blinded to both the mRDT and each other's microscopy result. In case of discordance the samples were subjected to a third and decisive *expert microscopy reading* conducted at Karolinska Institutet.

Filter papers were dried, stored and transported to Karolinska Institutet after field study completion. After DNA extraction, all RDT positive samples were analysed with three *P. falciparum specific nested PCR methods*[205] [206]. The RDT negative samples were screened in duplicate for human plasmodial infection with an *18s quantitative PCR* with minor modifications[207]. Samples with discrepant RDT and PCR results were subjected to confirmatory nested PCR analysis targeting *Plasmodium* Cytochrome b[208]. Samples with a negative mRDT but positive microscopy/PCR were subjected to PCR analysis of HRP-2 deletion[209].

Specific methods, study 2

Study design and participant recruitment, study 2

In study 2, 2-59 months old children were included that had an acute uncomplicated febrile illness (by history and/or electronic axillary temperature ≥37·5°C) and were seeking care at a PHCC (Kivunge Cottage hospital) in Zanzibar from April to July 2011. *Exclusion criteria* were signs of severe disease (according to IMCI); previous study enrolment in the last 28 days; and reported inability to return for follow-up.

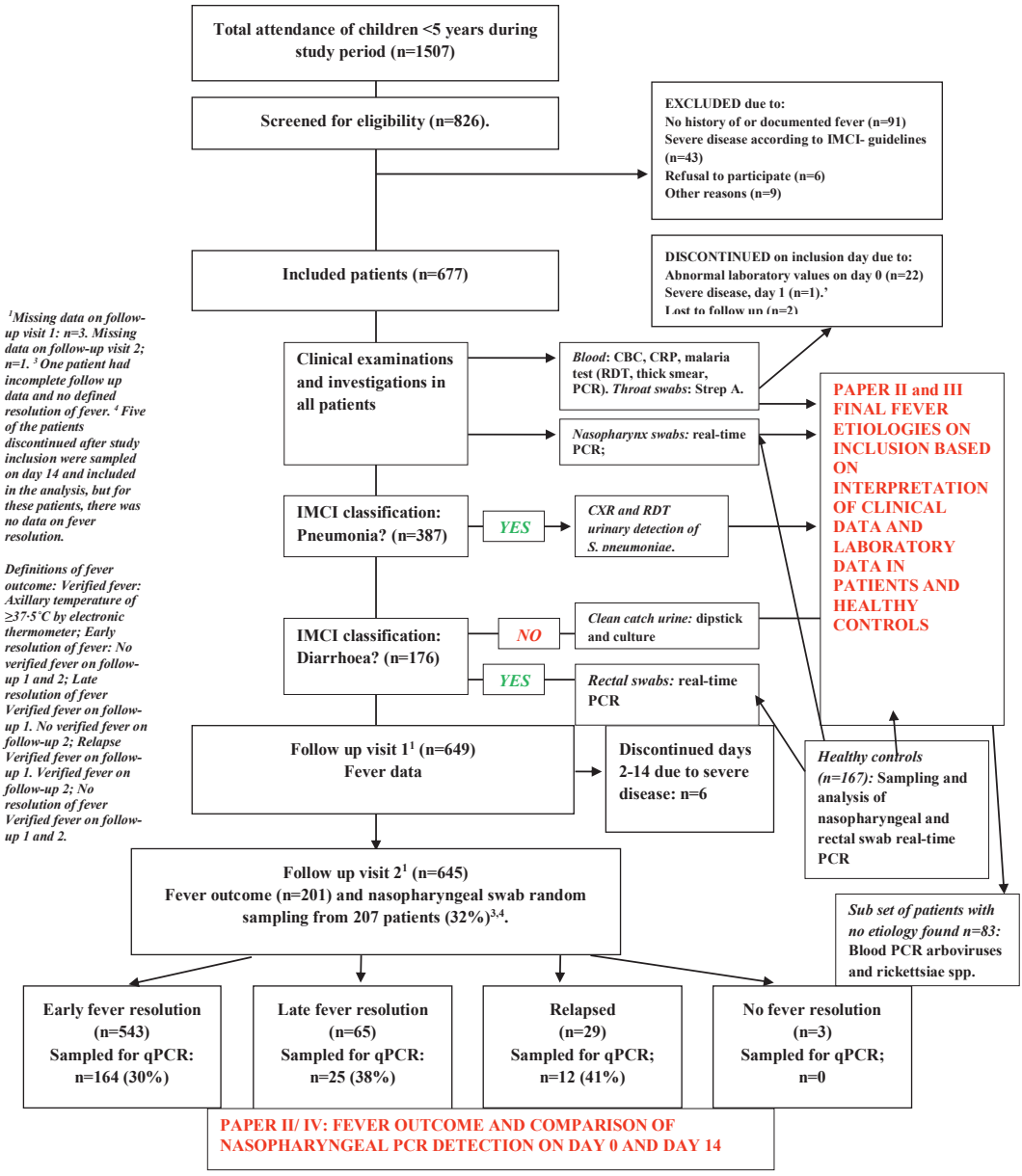
After enrolment, patients were subjected to *IMCI management and treatment* including an mRDT (SD Bioline-Pf/Pan®). Data was documented in a structured IMCI-based questionnaire. There after *pre-defined study investigations* were performed like POC tests, urine cultures, as well as multiplex real-time PCR investigations of nasopharyngeal and rectal swabs. Some were conducted in all patients and others in patients with certain clinical features identified through IMCI management.

In patients with pneumonia according to the IMCI guidelines, a confirmatory chest X-ray investigation was performed to diagnose radiological pneumonia (endpoint consolidation). Chest X-rays were performed, digitalised and interpreted according to WHO-standards with two independent radiologists blinded to each other's results, with a third and decisive expert reading if discordant results[148]. If suspected severe disease on CXR, patients were referred to a paediatric specialist.

During the field study, recruitment of 167 age- and geography- matched healthy controls was performed, defined as children aged 2-59 months with no history of diarrhoea, cough, running nose or fever (by history and/or electronic axillary temperature <37·5°C) in the preceding ten days. Eligible children, maximum two per household, provided nasopharyngeal and rectal swabs for real-time PCR-analyses.

Figure 4 shows the flow of patients in study 2 (*paper II-IV*) and summarizes all laboratory and radiology examinations performed on patients and healthy controls in study 2. Two visits were scheduled during the *14-day follow-up* and guardians were instructed to come back for unscheduled visits if the condition deteriorated. On follow-up day 14, 25% of patients were randomly selected for paired (day 0 and 14) nasopharyngeal swab sampling.

Figure 4. Study flow of patients in study 2 (paper II-IV)



[1]Missing data on follow-up visit 1: n=3. Missing data on follow-up visit 2; n=1. [3] One patient had incomplete follow up data and no defined resolution of fever. [4] Five of the patients discontinued after study inclusion were sampled on day 14 and included in the analysis, but for these patients, there was no data on fever resolution.

Definitions of fever outcome: Verified fever: Axillary temperature of ≥37·5˚C by electronic thermometer; Early resolution of fever: No verified fever on follow-up 1 and 2; Late resolution of fever Verified fever on follow-up 1. No verified fever on follow-up 2; Relapse Verified fever on follow-up 1. Verified fever on follow-up 2; No resolution of fever Verified fever on follow-up 1 and 2.

Laboratory management, study 2

All laboratory analyses were performed and interpreted according to the manufacturer's instructions. Malaria analyses were identically performed as in study 1 except that in study 2 the mRDT deployed by the time of the study the ZMCP was SD- Bioline-Pf/Pan® detecting *P. falciparum* specific HRP-2 antigen and pLDH-Pan.

In study 2, all patients had malaria RDT, BS and PCR performed. The following laboratory tests were performed on day 0, of which some were followed by a study intervention. Also, participants with pre-defined abnormal laboratory results and/or signs of severe disease were discontinued and referred for further clinical management (*intervention*) as described in more detail in *paper II*.

Results available at the point-of-care on day 0

- **Throat (swabs)**: an RDT for qualitative antigen detection (positive/negative) of GAS (QuickVue® Dipstick- Strep A test). *Intervention:* A patient with a positive test received penicillin V if not already treated with equivalent antibiotic.
- **Whole blood (capillary finger prick):** Malaria RDT (SD Bioline *Pan/Pf*®), and CRP (Nycocard®) were performed, the latter with an analytical range of 8-200 mg/L. *Intervention:* Abnormal CRP result: >200 mg/L
- **Urine (clean catch):** Semi-quantitative biochemical detection of leukocyte esterase, nitrite, albumin and erythrocytes. (CombiScreen® (7 SYS PLUS)). Rapid detection of pneumococcal antigen in urine (BINAX NOW®). *Intervention:* none

Results available within a couple of days after inclusion

- **Whole blood (capillary finger prick):** Malaria microscopy (first reading), Complete Blood Count (CBC) (Sysmex® KX-21). *Intervention:* BS positive for malaria, not previously detected received antimalarial treatment. *Intervention:* Abnormal laboratory result: white blood cell count >35*10^9/L; platelet count: <20*10^9/L; Haemoglobin: <6 g/dL

- **Urine (clean catch):** Preserved in 2-8°C, sent for culture to the microbiology laboratory and followed WHO standards[210 211]. *Intervention:* Patients with positive urine cultures were treated with an antibiotic corresponding to the susceptibility pattern.

Test results available after field study completion

- **Urine culture:** Colonies from urine cultures with significant growth sent for external quality control for verification of species and antibiotic susceptibility pattern. (Gothenburg, Sweden)
- **Whole blood/dried blood spots:**
  - *Detection of malaria:* Equal to study 1. Malaria microscopy (second and third reading) and PCR. (Zanzibar/Stockholm, Sweden).
  - *Detection of Rickettsia spp: Quantitative real-time reverse transcription PCR (qRT-PCR)* by using a *Rickettsia* genus-specific qRT-PCR targeting the *gltA* gene of Spotted fever group *Rickettsia spp* and an *R. felis*-specific qRT-PCR targeting the *bioB* and *orfB* genes[212]. qRT-PCR to detect Typhus group *Rickettsia* spp. targeting the hypothetical protein encoding gene[213]. (Aix, France)
  - *Detection of dengue virus (DENV), West Nile virus (WNV), Rift Valley fever virus (RVFV), and chikungunya virus (CHIKV) by PCR:* RNA was extracted from DBS (n=84) and screened by a beta actin PCR for RNA quality control. The dengue PCR semi-nested PCR has been published by Harris et al[214]. In house real-time PCRs were used for detection of RVFV, WNV, and CHIKV[215]. (Stockholm, Sweden)
- **Nasopharyngeal and rectal swabs:**
  - **Sampling:** Flocked nasopharynx and rectal swabs were sampled and preserved in a standardised manner and stored in a controlled temperature of <-70°C.
  - **Real-time PCR:** Automated nucleic acid extraction was followed by multiplex, multi-targeting real-time PCR amplification of the following respiratory pathogens: adenovirus, bocavirus, coronavirus (229E, OC43, HKU1,

NL63), enterovirus, metapneumovirus, influenza virus (A and B), measles virus, parechovirus, parainfluenza virus (1-3), respiratory syncytial virus (RSV), rhinovirus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Streptococcus pneumoniae*[216 217] and the following diarrhoeagenic pathogens: adenovirus, adenovirus (40/41), astrovirus, norovirus genogroup 1 and 2 (GG1 and GG2), rotavirus, sapovirus, *Campylobacter jejunii*, Enterotoxigenic *Escherichia coli* (ETEC)-*estA*, ETEC-*eltB*, *Salmonella spp*, *Shigella spp*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Cryptosporidium parvum/hominis*. For further analysis, the PCR results were recorded as detection (positive or negative) and for reactive samples also as the Ct (threshold cycle) value.

- **Sequencing of enterovirus/rhinovirus in the nasopharynx:** When both enterovirus and rhinoviruses were positive and there was a >10 cycle difference in Ct values, the result was in favour of the pathogen with the lowest Ct value. Otherwise sequencing was performed for species identification[217]. Similarly, sequencing was performed if the Ct value was >4 cycles lower on day 14 than day 0 (indicating a probable new infection)

Data management and statistical analysis

Sample size calculations and data entry

Sample size calculations were performed in study 1 based on the primary endpoint, adherence to mRDT results with the assumption that around 10% of the RDT negative patients would be prescribed ACT in Zanzibar. Study 2 was exploratory in nature, which precluded a sample size calculation. However, a sample of 650 patients and at least 150 controls was considered sufficient to obtain a representative classification of fever. After double *data entry* into CSPro, further validation of data was performed in Microsoft Excel®. Thereafter data was exported to STATA® (10, 12 and 14) where all statistical analyses were performed.

Statistical analysis study 1

P-values <0.05 were considered statistically significant. Frequencies, proportions and odds ratios (ORs) were calculated with 95% confidence intervals (CI) as appropriate. The fact that blood sampling for malaria microscopy and PCR among RDT negative patients only included a random sample of 20% was accounted for. Thus, the absolute numbers of observations were multiplied by the factor 5.14 and 5.01 in all calculations on the secondary endpoints that compared mRDT against microscopy and PCR (sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV).

Statistical analysis study 2

P-values <0.05 were considered statistically significant. Frequencies, proportions and odds ratios (ORs) were calculated with 95% confidence intervals (CI) as appropriate. For independent samples, Fisher's exact test and exact binomial test were used for binary data and proportions, two-sample t-test for mean comparisons, and Mann-Whitney-U test for median comparisons. The association between CXR-confirmed pneumonia and multiple variables was assessed with logistic regression. The cut-off values for inflammatory biomarkers (CRP and white blood cell count (WBCs)) were chosen to concur with published literature[218]

(*paper II and III*). In *paper IV*, detection and pathogen load (Ct value) were analysed by comparing paired multiplex real-time PCR data on nasopharyngeal swabs sampled from a sub-group of randomly selected patients in the parent study collected on study inclusion (day 0) and on a clinical follow-up after 14 days. The exact sign tests were used for binary data and paired t-test for the comparison of means.

Establishing a Ct cut-off value

The PCR results from the nasopharyngeal (*paper II*) and rectal swabs (*paper III*) collected from patients and healthy controls were analysed. In addition to comparing detection rate proportions and corresponding ORs, the potential utility of pathogen load in terms of real-time PCR threshold cycle (Ct) values to separate symptomatic from asymptomatic infections was evaluated. The Ct value is inversely related to the pathogen load in each specimen. For some pathogens, a Ct value cut-off was identified that optimally distinguished between patients and healthy controls, as follows.

Ct values from nasopharyngeal (*paper II, supplement*) and rectal qPCR (*paper III*) were compared in a logistic regression with patient/control as dependent variable and age (continuous), and gender (binary) as well as Ct values (continuous) as multiple independent variables. This analysis first included Ct values for all agents (with negative variables given Ct value 45). Factors not independently associated with symptoms were omitted in a step-wise manner. The cut-off was determined by using the highest balanced accuracy value (BCR) defined as follows: (BCR=$0.5 \times$ *(sensitivity + specificity))*. Agents independently associated with disease were classified as *more likely causes of fever*. Independent agents had either a difference in crude detection rate between patients and controls or proportions with Ct values below the above defined Ct value cut-off

Determining final cause of fever and infections that require antibiotics

Individual *final patient diagnoses or causes of fever* were retrospectively established by two paediatricians based on all available clinical and laboratory data in order to evaluate the utility of IMCI for identification

of infections presumed to require antibiotics. The final diagnoses were generated from pre-defined criteria based on available clinical and radiology/laboratory data from day 0. Subsequently, these criteria were applied to each patient.

The diagnoses were categorized into three groups: 1) more probable causes of fever, 2) less probable causes of fever, and 3) "no verified aetiology". If a more probable diagnosis was identified, all less probable diagnoses were ignored for that patient, but within one group each patient could receive more than one diagnosis. Multiple final causes of fever were possible. *Infections requiring antibiotics* were defined retrospectively as those final diagnoses presumed to benefit from antibiotics based on WHO recommendations[219]. The association between these infections and both actual antibiotic prescription as well as IMCI indication for antibiotics was assessed.

RESULTS AND DISCUSSION

Malaria prevalence during and after the rain season in Zanzibar

Out of 20423 patient consultations performed in the 14 included health facilities, 3890 (19%) were included in study 1 of which 1822 (46,9%) were children <5 years of age and 528 (13,6%) were 5-14 years of age. Hence, more than 60% of the included children were aged below 15 years which corresponds fairly well with the demographic structure of Zanzibar where official statistics claim that 45% of the population are below 15 years old.

In study 2, out of 826 children screened for eligibility, 677 children were enrolled of which 42% (286/677) had a measured axillary temperature of ≥37.5°C, similar to *paper I*, which had a corresponding figure of 43% (1539/3550). (Figure 4). These results are similar to previous research where 30-80% of patients with a reported fever actually have a measured temperature at the time[24 42-46].

Both study 1 and 2 were conducted during the main malaria season in Zanzibar, which occurs in the end and directly after the rain season. Yet, malaria prevalence by mRDT among febrile children <5 years of age was low, 2.0% (36/1822) and 0.3% (2/677) in *paper I* and *II*, respectively. These results are in line with a recent paper by Björkman, Shakely et al showing data from Zanzibar where in 2010, positivity rate among patients tested at the PHCUs for malaria in North A and Micheweni was 0.8% and 1.7%, respectively in 2010 and 0.4% and 0.8%, respectively in 2011 which also were the years that study 1 and 2 were conducted. Moreover, in 2002, half of the malaria positive patients were below 5 years old while the same proportion was 17% in 2015[19].

Overall positivity rate of mRDT was 121/2889 (3.1%). mRDT positivity was significantly associated with both history of travel (OR 3.27) as well as absence of ITN/LLIN use (OR 4.78). RDT positive children had significantly higher axillary temperature than RDT negative patients (38.3°C versus 37.4 °C; $p<0.001$). Children aged 5-14 years had the highest malaria prevalence by mRDT of 6.1% (32/528) (*paper I*), in comparison with the overall malaria prevalence; 3.1% (121/3889). These factors indicate the demographic transition where the highest malaria positivity rate is seen in school aged children rather than younger children do in Zanzibar and other African countries[19 220]. The reasons are probably mainly behavioural such as the fact that that older children spend more time outdoors in the evening and are thus less prone to lie under a mosquito net. Older children also travel more than younger do which is a risk factor for malaria infection in both mainland Tanzania[102] and Zanzibar[19].

Malaria control interventions focus primarily on protecting the preschool children from malaria since they are the most vulnerable. Interventions like for example bed net distribution are probably more effective in younger than in older children. If targeting malaria infections in school children, other new or established intervention strategies might be considered, like for example mass testing and treatment (MTAT), or preventive chemotherapy like mass drug administration (MDA) and vaccination[220]. However, in two recent randomised controlled trials conducted in Zanzibar neither MTAT[221] nor MDA[222] had an impact on overall transmission.

Malaria RDT test performance.

In study 1 the HRP-2 based mRDT (Paracheck®) performed mediocrely with a sensitivity of 76.5% (95% CI 69.0–83.9%) against PCR and 78.6% (95% CI 70.8–85.1%) against microscopy (expert reading). Specificity was >99%. The diagnostic performance was below the result from the 2009 WHO report that compared different brands for malaria RDTs (round 2). It showed a good sensitivity (97.5%) of Paracheck® for high parasitaemias but only 54% for parasitaemias at 200 parasites/uL[223]. One reason for false negative results could be the changes in the genes that code for the HRP-2 protein, which is the malaria antigen detected in the mRDT[224]. In study 1, all samples with discordant results between mRDT and microscopy and/or PCR were screened, but no so-called *P. falciparum HRP-2 deletions* were detected. In contrast, a recent report from Eritrea showed that 80% of the parasites detected had these HRP-2 deletions and subsequently most of them were also HRP-2 RDT negative[225].

Another reason for a false negative test is the so called *prozone effect* which is a false negative result due to an excess of malaria antigen that exists for HRP-2 based tests only, since three patients with high parasitaemia on microscopy were mRDT negative[226], although the prozone effect has mainly been described for hyperparasitaemias (defined as >250 000 parasites/uL or 5% of the erythrocytes infected), which none of the patients included in study 1 had. The prozone effect has not been shown for pLDH or aldolase mRDTs[227].

The last and most probable reason is the operator factor. Most of the mRDT negative and microscopy positive patients came from the same health facility. This further sheds light on the fact that for all types of interventions, supervision and training is key[228 229]. The low sensitivity in our study is in contrast to research by Batwala et al who showed that HRP-2 based RDTs like Paracheck® outperform both health centre malaria microscopy as well as expert microscopy in sensitivity and specificity, both in high endemic and low endemic settings[230-232]. Others have confirmed the results of study 1 with a low sensitivity of HRP-2 RDTs and conclude that in an area where malaria is decreasing, HRP-2-

based detection of malaria is suboptimal[233] due to an unacceptable high number of false negatives[79] whereas false positive mRDT results are more of a problem in high endemic areas[78 234]. The false positive tests might be due to the well-known prolonged antigenaemia detected by the HRP-2-based tests for up to a month after a *P. falciparum* infection[235].

Out of the 124 patients that were positive for P. falciparum malaria by PCR, 9 infections were not found with malaria microscopy (*paper I*). Ramesh et al also recently published an article on children with fever of unknown origin in Uganda, which showed that around 50% of patients had malaria detected with metagenomic techniques whereas only 10% were positive by microscopy[236]. This could call for more sensitive molecular techniques to replace microscopy as gold standard reference in malaria elimination settings as some research groups propose[231].

Yet, many children residing in high malaria endemic areas may have asymptomatic parasitaemia with doubtful clinical relevance. Some argue that the level of parasitaemia that defines a clinical malaria case should be different depending on both the age of the patient as well as the level of malaria endemicity in the area where the patient resides. The lower the malaria prevalence, the lower the threshold for regarding a parasitaemia a true infection. Also, low parasitaemias may play a more important role in spreading the infection than previously thought in lower endemic or pre-elimination areas[73 74]. Hence, in research studies in high endemic areas microscopy could be preferable as gold standard whereas more sensitive tools like PCR might be better in low endemic settings.

Despite the mentioned disadvantages of mRDT, most experts nowadays would still advocate the use of mRDTs before health care facility microscopy in routine clinical care[228]. Furthermore, the mRDT in study 2 performed well for identification of *P. falciparum* where there was a 100% concordance between the results of PCR, BS and mRDT. Importantly, there were no false positive mRDT tests in the malaria negative patients. This further supports the introduction of the SD Bioline® Pan/Pf which the Ministry of Health in Zanzibar decided on based on the results from

study 1. Also, the test performance of the SD Bioline® Pan/*Pf* in round 2 of the WHO report from 2009 was excellent[223].

The finding that *P. malariae* might play a greater role could also be a retrospective support for the SD Bioline® Pan/*Pf* introduction since that test is also detecting non-falciparum malaria. Importantly, two out of 116 malaria infections in *paper I* were positive for *P. malariae* by PCR. The proportion of *P. malariae* in the clinical and asymptomatic malaria infections in East Africa have increased in the last decade[19 237]. *P malariae* is an infection that could remain dormant in blood cells for a long time (years). Therefore it may constitute a higher share of the detected parasites in blood. Especially if investigations include PCR that could detect low parasitaemias below the threshold of both microscopy and malaria RDT. Also the fact that co-infections between *P. falciparum* and other species like *P. ovale* and *P. malariae* are common might indicate that these infections were underdiagnosed before[237]. A recent report highlights the low performance of existing mRDTs to detect non-falciparum species, in particular *P. malariae* and *P. ovale* [238]. With increasing proportions of non-falciparum species among malaria patients, a higher and more coordinated focus on their diagnosis and management is needed.

Health worker adherence to malaria RDT results

Paper I showed that health worker adhered to mRDT results in 99.9% of cases, both with or without the IMCI guideline. Antimalarial treatment was prescribed to all mRDT positive patients but only to 3/3768 (0.08%) of patients that had a negative mRDT. This high adherence rate is in contrast to other similar studies, that exhibit lower adherence to both negative and positive mRDTs[239], in some as little as 80% of mRDT positive patients and over 30% of mRDT negative receive antimalarials[240].

A meta-analysis of similar studies showed that health providers were more likely to adhere if the result was according to their expectations. Adherence was also higher if the RDT had already been integrated into standard care and if there was a clinical management strategy for the mRDT negative patients[229]. Zanzibar has in the recent two decades had a comprehensive and versatile malaria control campaign, which probably is one explanation for this.

Indeed, scientists argue that the great problem in malaria endemic settings is the overprescription of antimalarials. However, underprescription is likely underestimated[240]. It also matters where the test-and-treat package is implemented. For example, RDTs introduction to a part of the private drug shop sector in Ghana was successful and increased accurate antimalarial treatment from 27% to 74% in the intervention arm where patients were provided free mRDTs[241]. Specific studies on the performance of mRDT integration within the IMCI have rarely been reported before[65]. mRDT integration within the IMCI did not affect adherence in our study, and thus was optimal.

A false positive mRDT is sometimes regarded a substantial clinical risk by misleading the clinician away from diagnosing the co-existing bacterial infection[242]. However, the opposite has also been shown. In Senegal, the National Malaria control programme introduced a new fever

management protocol in 2007 that guided the health care worker in a febrile patient to first consider "other causes of fever" like upper respiratory tract infections (URTI) or pneumonia. The results of a trial attached to this intervention showed that the guideline excluded too many patients with malaria from receiving an mRDT at their first visit[243], most likely due to the fact that the clinical picture of malaria is typically unspecific and can include both respiratory tract and gastrointestinal symptoms.

The PRIME trial evaluated the effect of a patient centred health intervention package with the aim to increase quality of care and better outcomes[244]. It showed that accurate antimalarial prescription was similar in the intervention arm and the control arm with almost a fourth of the patients with a positive malaria test that did not receive antimalarials[245]. The above mentioned reports exemplify that designing a clinical fever management intervention is complicated and poses a risk of undertreatment with antimalarials.

Distinguishing between cause of illness and asymptomatic infection

Study 2 was the first comprehensive study on non-severe febrile illness in children in Africa using a healthy control group for comparison of PCR results. A total of 22257 analyses were conducted for the detection of 36 pathogens, of which a majority was detected by using molecular analyses. Figure 4 shows the flow of patients in study 2. Median detection was three pathogens (range 0-10) per patient and only eight patients had no microbe detected. There was a high detection rate of pathogens detected by real-time PCR both in the 677 included patients and the 167 healthy controls (Table 1).

The crude detection of a pathogen nucleic acid in a sample does not always indicate cause of disease. This is of particular importance in low-income countries, where children due to poor sanitary conditions or overcrowding are more likely exposed to multiple pathogens. Thus,

understanding aetiologies and interpretation of test results require analysis of pathogens from both ill and healthy individuals. In the recent decade four large multicentre studies from LMICs on infectious disease aetiology in children below 5 years of age that use molecular techniques and also include a control group. Two study diarrhoeal disease; firstly the Global Enteric Multicentre Study (GEMS) on moderate to severe diarrhoeal disease[20] and secondly the multi-country Malnutrition and Enteric disease (MAL-ED) study that analysed enteropathogen contribution to community diarrhoeal disease in the first two years of life by comparing diarrhoeal and non-diarrhoeal samples in a longitudinal birth cohort[246 12]. Both GEMS and MAL-ED later re-analysed their samples using quantitative measures of PCRs[15 178 186].

Two other studies report the causes of severe/hospitalized pneumonia in patients with WHO defined endpoint radiological pneumonia; the study from the GABRIEL-network (Global Approach to Biological Research, Infectious diseases and Epidemics in Low-income countries)[16] as well as the Pneumonia Etiology Research for Child Health (PERCH) study (published in July 2019)[13 14]. All these studies use the population attributable fraction (PAF) to define the contribution of each pathogen to the overall load of illness in the population studied[12 14 180 202 246] and similar to study 2 the results from the PERCH study also will define a final diagnosis for each individual patient[203].

Respiratory tract pathogen detection in patients and healthy controls

Real-time PCR conducted on nasopharyngeal swabs showed that 98% of patients and 93% of controls were positive for one or more pathogens (*paper II*). More than one pathogen was detected in 88% of sampled patients and 83% of sampled controls.

Influenza A and B virus, RSV and enteroviruses were significantly more common in patients than controls, with the opposite for rhinovirus and coronavirus which were more common in controls (Table 1).

Other studies from the African region comparing healthy controls and patients with fever and/or respiratory symptoms like URTI and pneumonia have shown similar results with high detection rate in healthy individuals[13 16 247-249]. From previous literature, the pathogens that have emerged as significantly more common in cases than controls are influenza A and B virus, metapneumovirus, parainfluenza virus and in particular RSV[13 16 180 247-255]. For adenoviruses, bocavirus, rhinoviruses and coronaviruses, which are often common in both cases as well as controls there are conflicting results.

Some aetiology studies choose not to include the analysis of rhinoviruses at all[248]. Despite this, both the GABRIEL and PERCH studies point out rhinovirus as a common cause of hospitalised pneumonia[13 16]. Also, the GABRIEL study had a strong association with being a case for influenza A (adjusted OR 55) in line with the results from *paper II* (Table 1; OR 5.1). Interestingly, the PERCH study could not show the same strong association since there was a generally low detection (<5%) of influenza A and B viruses.

Table 1. Real-time PCR pathogen detection and Ct values on nasopharyngeal and rectal swabs from patients and healthy controls. Modified from *paper II and III*.

Pathogen	Patients n positive (%)	Controls n positive (%)	Unadjusted OR	P[a]	Ct value median (patients/ controls)	P[b]
Nasopharyngeal swab PCR (n sampled)	**672**	**166**				
Viruses						
Adenovirus	12 (1.8%)	1 (0.6%)	3	0.48	29.5/36.6	0.42
Bocavirus	20 (3.0%)	4 (2.4%)	1.2	1.0	36.7/38.3	0.19
Coronavirus	52 (7.7%)	23 (14%)	0.5	0.02	31.9/31.7	0.33
Enterovirus	58 (8.6%)	1 (0.6%)	15.6	<0.0001	36.0 /35.4	0.81
Metapneumovirus	11 (1.6%)	0 (0%)	.	0.13	28.6/0	.
Influenza B virus	94 (14%)	4 (2.4%)	6.6	<0.0001	27.8/34.7	0.0083
Influenza A virus	58 (8.6%)	3 (1.8%)	5.1	0.001	27.3/39.0	0.35
Morbillivirus	5 (0.7%)	0 (0%)	.	0.6	37.8/0	.
Parechovirus	5 (0.7%)	0 (0%)	.	0.6	32.6/0	.
Parainfluenza virus	10 (1.5%)	1 (0.6%)	2.5	0.7	24.9/23.8	0.75
Respiratory syncytial virus (RSV)	174 (26%)	27 (16%)	1.8	0.008	26.8/33.7	0.0025
Rhinovirus	105 (16%)	48 (29%)	0.5	<0.0001	30.7/28.7	0.022
Bacteria						
Bordetella pertussis	5 (0.7%)	0 (0%)	.	0.59	30.2/0	.
Chlamydophila pneumoniae	8 (1.2%)	0 (0%)	.	0.37	40.4/0	.
Haemophilus influenzae	515 (77%)	130 (78%)	0.93	0.76	30.2/29.4	0.023
Mycoplasma pneumoniae	2 (0.3%)	1 (0.6%)	0.5	0.49	38.2/37.9	1
Streptococcus pneumoniae	587 (87%)	137 (83%)	1.5	0.128	27.3/26.9	0.08
Rectal swab real-time PCR (n sampled)	**164**	**165**				
Viruses						
Adenovirus (any)	45 (27%)	53 (32%)	0.79	0.4	38.2/39.3	0.05
Adenovirus 40/41	10 (6.1%)	6 (3.6%)	1.71	0.44	36.6./35.0	0.66
Astrovirus	4 (2.4%)	1 (0.6%)	4.07	0.37	19.9/31.5	
Norovirus GI	1 (0.6%)	1 (0.6%)	1			
Norovirus GII	33 (20%)	4 (2.4%)	10.1	<0.0001	25.1/26.9	0.28
Rotavirus	16 (9.8%)	3 (1.8%)	5.8	0.003	24.4/26.0	0.5
Sapovirus	13 (7.9%)	7 (4.2%)	2.09	0.18	25.6/28.3	0.5
Bacteria						
Campylobacter	58 (35%)	54 (33%)	1.11	0.73	31.8/33.3	0.12
Vibrio cholerae	1 (0.6%)	0 (0%)		-		
ETEC-*eltB*	71 (43%)	76 (46%)	0.91	0.74	31.3/34.6	0.002
ETEC-*estA*	54 (33%)	39 (24%)	1.62	0.07	32.6/37.3	0.0001
Salmonella	9 (5.5%)	4 (2.5%)	2.32	0.26	42.2/40.6	0.22
Shigella	56 (34%)	54 (33%)	1.08	0.82	29.2/34.5	<0.0001
Yersinia	0 (0%)	0 (0%)				
Protozoa						
Cryptosporidium	49 (30%)	18 (11%)	3.45	<0.0001	32.1/36.8	0.0009

ETEC: Enterotoxigenic *Escherichia Coli*; OR, odds ratio; PCR: Polymerase Chain Reaction; RDT: Rapid Diagnostic Test [a] Fisher's exact test.

[b] Mann-Whitney U test displayed for agents with a total of >10 positives samples.

Enterovirus was shown to be undoubtedly associated with fever in study 2 (Table 1; OR 15.6). Hercik et al reported enteroviruses in 15% of their febrile patients[185] but besides enteroviruses have rarely been reported from fever studies in Africa. One reason could be that enteroviruses normally do not cause respiratory symptoms but are instead the cause of undifferentiated fevers[256].

Most previous studies that apply nasopharyngeal PCR include patients with acute respiratory but not fever without respiratory symptoms. In agreement, rhinovirus does not commonly elicit a fever and therefore can be more common in afebrile patients. Also enteroviruses could cross react with rhinoviruses in the real-time PCR reaction due to the RNA sequence similarities. In previous studies, it is often unclear how enteroviruses and rhinoviruses are distinguished.

In study 2 separate real-time PCR assays were used for enterovirus and rhinovirus, and sequencing was performed for further characterization if the Ct value difference was less than ten cycles. The results showed that the most common rhinovirus type was type C (12 out of 15 patients (80%) with final result rhinovirus). Human rhinovirus type C was discovered in 2007[257] by the application of molecular techniques (it is difficult to propagate in cell cultures) and might be associated with more severe symptoms than other rhinoviruses. Recent research in the SSA region show that rhinoviruses are common and in particular type C using both real-time PCR techniques[258] and metagenomics[236].

A limitation of study 2 was the age difference between healthy controls and patients. We decided to include healthy controls from the community and not from the same health facility where patients are recruited, as some studies choose to do[16]. This was to make sure that recruitment of healthy controls was not impeded by a lack of participants and to get a geographically representative sample from the whole catchment area of the health facility. However, the limitation was subsequently that the controls recruited were median 10 months older than the patients (*paper II-IV*), which may have been avoidable if we had actively recruited from the health facility where the study was conducted.

Another important question when choosing control subjects for studies on infectious disease aetiologies, is whether controls should be allowed to be symptomatic or not. Study 2 included only asymptomatic controls both for rectal swab and nasopharyngeal sampling in line with the GABRIEL and GEMS studies. There is a risk of selection bias, since it could particularly be difficult to recruit infants without a history of fever, ARI symptoms or diarrhoea in the last 2 weeks. Therefore, as decided in the PERCH study, community recruited controls with respiratory symptoms could be regarded as more representative for the general population as long as they do not qualify to be a case[259]. This risk might be more obvious in a study on pneumonia than fever.

Influenza B and RSV were more common in patients than healthy controls and patients also had significantly lower median Ct values (Table 1). Interpretation of Ct values as a proxy for pathogen load of simultaneously detected multiple pathogens had not systematically been performed before the conduct of study 2, and in particular not in LMICs. However, a distinct Ct value cut-off to distinguish disease from asymptomatic infections could not be defined for any nasopharyngeal agent why all detected pathogens (with Ct value <40) were included in the analysis.

The PERCH study which included children 1-59 months with severe and very severe pneumonia according to the WHO definition, recently reached a similar conclusion. It showed that there was a significant difference in mean Ct value for adenovirus, parainfluenza virus 1 and 3, RSV, and rhinovirus[253] between the following groups; asymptomatic controls, patients with respiratory signs and pneumonia cases. However, due to substantial overlap in viral load distribution a cut-off to distinguish between pneumonia and upper respiratory tract infection could not be identified for any virus.

Diarrhoeal pathogen detection and Ct value cut-offs

It is sometimes argued that patients with gastroenteritis should not be included in a study on fever aetiologies. Yet, we considered it important to include patients with diarrhoea as well. First of all, diarrhoea is the second largest killer in children younger than five years[260]. Secondly, we thought that patients with diarrhoeal disease should not be excluded considering the exploratory nature of study 2 for it to be as unbiased as possible and include the whole spectrum of patients that a clinician encounters on the first level of health care.

Indeed, in the MAL-ED study described above, fever was the symptom associated with the most important diarrhoeal pathogens and could then be regarded as a marker of more severe illness[12]. A fourth of the patients (n=176) in our study had diarrhoeal symptoms which is in concordance with other clinical studies in the area[158]. Of these 176 patients, 63% (n=111) reported ≥3 stools/24 hours which was also the criterion for a gastroenteritis diagnosis in the interpretation of final diagnosis.

In *paper III*, we analysed the real-time PCR-results on rectal swabs from children with or without diarrhoea. Similarly to nasopharynx PCR, a high detection rate was seen with 95% of patients and 87% of controls that were positive for one or diarrhoeal more pathogens. More than one pathogen was detected in 36% of the 164 sampled patients and 30% of the 165 sampled healthy controls. *Cryptosporidium*, rotavirus and norovirus GG2 were significantly more common in patients than controls.

In addition to comparing detection rates, and similar to the analysis performed for nasopharyngeal PCR, we aimed to evaluate the potential utility of pathogen load in terms of real-time PCR threshold cycle values to separate symptomatic from asymptomatic infections. *Cryptosporidium*, ETEC *eltB,* ETEC *estA* and *Shigella* patients had a significantly higher median Ct value (pathogen load) than controls.

In contrast to nasopharynx PCR, a cut-off in Ct value could be defined by a two-step analysis for rectal swab PCR. The pathogens considered most important causes of diarrhoea, when frequencies and Ct value cut-offs in patients versus controls were applied were *Cryptosporidium* (Ct cut-off <35), *Shigella* (Ct cut-off <30), norovirus GG2 (Ct cut-off <40), ETEC-*estA* (Ct cut-off <31), and rotavirus (Ct cut-off <40).

When the cut-offs were used, the five most important diarrhoea agents among patients were *Cryptosporidium* (25%), *Shigella* (20%), ETEC-*estA* (16%), norovirus GG2 (19%), and rotavirus (9.1%). Ct values of 40-45 are normally the cut-off limit for the PCR- reaction but values in that range are often regarded too high to be clinically relevant and are potentially only finding the subclinical infections[187]. The Ct value cut-offs that emerge from this analysis are in the same range as previous studies. In general the cut-offs end up in between 30-35 for both diarrhoeal[187 188], as well as respiratory tract[198 199] pathogens and malaria[17 185].

The results from *paper II* and *paper III* were confirmed by the Global burden of disease study that in particular point out rotavirus as the most important pathogen and cause of death[6]. The GEMS study concluded that in addition to rotavirus preventive interventions specifically targeting *Cryptosporidium*, ETEC-*estA*, Enteropathogenic *E.coli* (EPEC) and *Shigella* would substantially reduce mortality and hospitalization of diarrhoea[15].

The MAL-ED study, that focused on diarrhoeal disease in the community which might be more comparable to the non-severely ill febrile patients in study 2, showed that norovirus GG2, rotavirus, astrovirus, *Campylobacter spp* and *Cryptosporidium spp* were the highest contributors to diarrhoeal disease in the first two years of life whereas *Shigella spp*, *Cryptosporidium spp* and ETEC-*estA* caused the most severe diarrhoeal episodes. Interestingly, in the sites where rotavirus vaccination was introduced, the contribution of rotavirus to diarrhoeal disease was negligible.

The finding that norovirus was one of the highest contributors to diarrhoeal disease both in MAL-ED as well as study 2 might support the

fact that noroviruses typically do not cause severe symptoms in children. Moreover, GEMS included more severely ill patients and noroviruses were not as common in that study. *Cryptosporidium* seems to be common but a previous study in Zanzibar found in contrast only a few patients that were infected. This highlights the need for correct choice of primers for the PCR reaction[261]. The primers that we chose[262] detect the most common species of *Cryptosporidium spp*, i.e. *C. parvum* and *C. hominis*[263]. In both the GEMS and the MAL-ED study, application of PCR increased the overall pathogen detection with 50-100% in comparison with traditional microbiology techniques that were used in the initial publications. Hereby, the detection of in particular shigellosis and sapovirus was increased with molecular techniques[178 186].

Causes of febrile illness

Study 2 was the first study in Africa on uncomplicated febrile illness that both applied a comprehensive laboratory panel as well as including a healthy control group. *Paper II-IV* show the result of a carefully thought through approach where in each individual patient a final diagnosis/cause of fever was determined (Table 2). Before this study-specific interpretation, the most common detected pathogens were *Haemophilus influenzae*, *Streptococcus pneumoniae*, RSV, influenza A or B virus and rhinoviruses in patients and *Haemophilus influenzae*, *Streptococcus pneumoniae*, rhinoviruses, RSV, and coronaviruses in healthy controls (Table 2).

Table 2. Final diagnoses versus pathogen detection in patients and healthy controls.

Pathogen	Patients n positive (%)	Controls n positive (%)	Final cause of fever n (%)
Total number patients/controls	**677**	**166**	**677**
Pathogen/diagnosis			
Respiratory syncytial virus (RSV)	174 (26%)	27 (16%)	166 (25%)
Influenza A/B virus	151 (22%)	5 (3.0%)	151 (22%)
Rhinovirus	105 (16%)	48 (29%)	71 (10%)
Enterovirus	58 (8.6%)	1 (0.6%)	58 (8.6%)
Group A Streptococci	89 (13%)	NA	45 (6.6%).
Radiologically verified pneumonia	42 (6.2%)	NA	42 (6.2%)
Coronavirus	52 (7.7%)	23 (14%)	30 (4.4%)
Shigella spp	55 (8.1%)	54 (33%)	29 (4.3%)
Cryptosporidium spp	49 (7.2%)	18 (11%)	26 (3.8%)
Caliciviridae	47 (6.9%)	12 (7.2%)	22 (3.2%)
Enterotoxigenic *E. Coli**	54 (8.0%)	39 (23%)	22 (3.2%)
IMCI Otitis	21 (3.1%)	NA	21 (3.1%)
Rotavirus	16 (2.4%)	3 (1.8%)	11 (1.6%)
Urinary tract infection/significant growth	16 (3.5%)	NA	11 (1.6%).
Metapneumovirus	11 (1.6%)	0 (0%)	8 (1.2%)
Bocavirus	20 (3.0%)	4 (2.4%)	8 (1.2%)
Adenovirus (ARI)	12 (1.8%)	1 (0.6%)	8 (1.2%)
Campylobacter spp	58 (8.6%)	54 (33%)	6 (0.9%)
Parainfluenza virus	10 (1.5%)	1 (0.6%)	6 (0.9%)
Bordetella pertussis	5 (0.7%)	0 (0%)	5 (0.7%)
Measles virus	4 (0.6%)	0 (0%)	4 (0.6%)
Chlamydophila pneumoniae	8 (1.2%)	0 (0%)	3 (0.4%)
Plasmodium falciparum	2 (0.3%)	NA	2 (0.3%)
Adenovirus 40/41 (GE)	10 (1.5%)	6 (3.6%)	1 (0.1%)
Parechovirus	5 (0.7%)	0 (0%)	1 (0.1%)
Measles virus	4 (0.6%)	0 (0%)	4 (0.6%)
Hemophilus influenzae	515 (77%)	130 (78%)	0 (0%)
Streptococcus pneumoniae	587 (87%)	137 (83%)	0 (0%)
Adenovirus (GE) non 40/41	45 (6.6%)	53 (32%)	0 (0%)
Vibrio cholerae	1 (0.6%)	0 (0%)	0 (0%)
Mycoplasma pneumoniae	2 (0.3%)	1 (0.6%)	0 (0%)
Astrovirus	10 (1.5%)	39 (23%)	0 (0%)
Salmonella spp	9 (1.3%)	4 (2.4%)	0 (0%)

* ETEC expressing either *estA* and/or *eltB*

After application of study specific pre-defined diagnostic criteria using clinical characteristics and laboratory results including the above described Ct value cut-offs in some pathogens, a cause of fever, was assigned to 579/677 (86%) patients of whom 160/579 (28%) had more than one final diagnosis. The most common causes of fever were RSV, influenza A or B virus, rhinoviruses, enteroviruses, and GAS (Table 2).

It is difficult to compare our study with previous reports on African children considering the substantial differences in methodology and inclusion criteria[158 159 264-266]. Indirect comparisons could be made by analysing large studies like the Global Burden of disease that include global estimations of mortality and morbidity from different pathogens. Yet, more important is probably to compare with aetiology studies like PERCH and GEMS that have other inclusion symptoms which indirectly cause fever (like diarrhoea and pneumonia).

Many previous fever aetiology studies are rather exploratory in nature and use new point-of-care tests, sensitive molecular analyses or sometimes more traditional techniques but might lack a comprehensive paediatric approach with a risk of selection bias behind the choice of investigated pathogens and their diagnoses.

A review from Kiemde et al[8] published just before *paper II* shows that most fever aetiology studies performed after the introduction of rapid diagnostic tests for malaria focused on febrile children under 5, included a mixture of inpatients and outpatients and mainly investigated presence of bacterial invasive infections but less so viral and parasitic infections. Also, of those that include viral infections most analyse only a few agents like adenovirus and rotavirus and influenza virus. Kiemde et al further illustrated the uniqueness of study 2 by pointing out that no other study analyses blood stream, urinary tract, gastrointestinal and respiratory tract infections simultaneously. They also emphasized the possible disadvantage to only study those with a measured temperature on-site.

Some recent studies have investigated the aetiology in each patient and thereby are easier to compare to study 2. The best example is the fever

aetiology study conducted in Tanzania by D'Acremont et al that applied a broad microbiology test panel, including PCR and POC diagnostic tests[161] and also retrospectively posed a final study diagnosis on individual level based on pre-defined criteria. I

In contrast to our study 2, they included older children with verified fever, both severe and non-severe cases, but did not include a control group of asymptomatic children. Their selection of analyses was also more complex with a study specific algorithm whereas study 2 used the information gathered from the standard of care in Zanzibar, the IMCI guidelines and was also less selective on which patients that should be tested. Importantly, results from nasopharyngeal PCRs were disregarded in the study by d'Acremont et al if there was another systemic (i.e. detected by serology or PCR in blood) viral, parasitic or bacterial infection detected. Hence, the most important cause of fever in study 2, a viral respiratory tract infection was deemed not as important in that study. Nevertheless, over 50% of their children had an upper respiratory tract infection as cause of fever with the following four top causes: Adenovirus, 27%, rhinovirus 21%, influenza A/B 19%, and bocavirus 11%[161]. However, study 2 showed that three of those mentioned pathogens were regarded as a less likely cause of symptoms and after the final interpretation our study considered merely 1% of the final diagnoses to be caused by Bocavirus and Adenovirus, respectively. We early on saw the need for inclusion of healthy controls, and without the reference of asymptomatic subjects, the conclusions on aetiology and final diagnoses in study 2 would not have been accurate.

Yet, d'Acremont's study team put more efforts into finding invasive bacterial infections and systemic viral infections like human herpes virus type 6 (HHV-6), cytomegalovirus (CMV) and Epstein-Barr virus (EBV). We did not include these pathogens in our study, which might be considered a study limitation. HHV-6 is indeed a well-known cause of fever in children especially in infants that often are infected during the first year of life typically with the clinical syndrome *exanthema subitum*. However, it is often difficult to determine whether HHV-6 PCR

positivity is caused by an acute infection, latent infection or asymptomatic reactivation without any clinical significance[267]. Also, it is difficult to distinguish between active replication and chromosomal integration into the host genome which is estimated to be present in around 1% of the population[268 269]. These difficulties are important to consider since HHV-6 was identified as the second most common cause of acute febrile illness in infants in the study by d'Acremont et al.

Fourteen percent of the patients in study 2 did not receive a final diagnosis. Yet, only 8 patients had no pathogen detected (*paper II*). The corresponding figure in the PERCH[13] and d'Acremont study were 1.8% and 3%, respectively. This difference could for example be explained by that both studies regarded a detection of pneumococci in the nasopharynx as a probable cause of disease whereas in study 2, we did not since 87% of patients and 83% of controls had pneumococci detected in nasopharynx (Table 1). Another reason could be that our study interpretation algorithm might have excluded some agents that were likely causing the symptoms. One example is norovirus GG2. Ten patients had a low Ct value (high pathogen load) for noroviruses but did not report three or more stools per day, which was a prerequisite for a pathogen found on rectal swabs to qualify as a final diagnosis. But in these circumstances, norovirus GG2 could have been a probable cause of disease since studies show that at least half of norovirus cases express fever and often abdominal pain and vomiting but no diarrhoea[270 271].

Another difference is that study 2 also included patients with only a history of fever, who comprised 58%. This might be seen both as a limitation -patients included are too well- but also as described above, a strength -patients represent those that should be managed according to the IMCI fever algorithm since it encompasses history of fever as well-.

Arboviral infections and other emerging infections

Indirect comparisons with research on travellers returning from Africa, both commercial tourists and so called VFRs (Visiting Friends and Relatives) have like study 2 shown that the majority of travel related fevers are caused by *cosmopolitan infections*, i.e. not "tropical diseases"[272-274]. However, in travelling children returning from tropical regions, the three most common causes of tropical diseases are not surprisingly, malaria, dengue, and enteric fever. Specifically, febrile returnees from sub-Saharan Africa had most commonly *P. falciparum* and rickettsioses whereas dengue fever was rare in epidemiological reports from 1999-2011[275 276]. Previous research indicates that rickettsioses are not as common in East Africa as in the Southern part, which can explain the fact that that no rickettsial infections were found in study 2[277]. Also, in study 2, no patients had an infection with dengue, chikungunya, Rift Valley fever virus or West Nile virus, which is in accordance to d'Acremont et al that did not find any arboviruses by PCR[161]. However, when using dried blood spots as in study 2, there is a risk for false negative results due to the degradation and instability of nucleic acids, in particular for RNA viruses (like chikungunya virus, dengue virus, West Nile virus, Rift Valley Fever virus).

Another limitation was the sampling selection, since study patients were only tested for blood pathogens other than malaria if no other cause of fever was detected. Also the time period when patients with arbovirus infections are viremic (PCR positive in blood) is often short. Still, the results are in coherence with other data from both Tanzania and Zanzibar conducted around the time of the study where there was no research nor official figures that reported acute or previous dengue infections in children below 15 years of age[278 279].

However, arboviral infections do exist in Africa and Zanzibar has been one of the first places to report dengue epidemics[280]. Studies indicate that dengue has been present in West Africa for at least 30 years[281-283]. Although dengue as a cause of febrile disease in Africa is increasingly reaching attention[284], previous research are case reports or retrospective,

often serology-based[285] blood donor data or studies performed in travellers that only indirectly identify the presence of dengue in Africa[286] [287]. Yet, how high the actual disease burden of dengue virus is in Africa and Tanzania is still unknown[283], and the need for dengue surveillance has been highlighted[280]. Recently, population based studies across Africa have been initiated[288] and some with preliminary reports of ongoing epidemic transmission[289-292]. In children, uncomplicated arboviral infections are common. Malaria and arboviral infections often have overlapping symptomatology and co-infections between malaria and dengue have also been reported[291]. Hence, dengue diagnosis particularly in small children is difficult.

Since the risk of serological cross-reaction is substantial, exposure to other flaviviruses than dengue, like zika virus, West Nile fever virus and tick-borne encephalitis virus often has to be considered. Attributing infectious disease burden in children based on serology results could be performed retrospectively[19 96] or prospectively[293], preferably by detection of IgG seroconversion, although detection of IgM on one occasion might be sufficient. If a sample is taken shortly -within 1-2 weeks- after debut of symptoms, dengue infections may be diagnosed by PCR-based assays or NS-1 antigen detection[95].

Chikungunya is an arbovirus that like dengue is transmitted by the Aedes mosquito. Chikungunya epidemics in Africa have been described in the last decade and are more common than dengue in aetiology studies on febrile illness in children[264 279].

The arboviral infections and also some of the other agents analysed on dried blood spots in study 2 are often classified as emerging diseases. Emerging and re-emerging infections are defined by the United States Centre for Disease Control as "infections that have increased recently or are threatening to increase in the near future". Many emerging infections increase due to for example over-population, migration and climate change[294].

However, just as important a reason for a reported increase may be the fact that many of these pathogens were previously difficult to detect in the laboratory. New and more sensitive laboratory techniques combining serology with pathogen detection techniques like PCR have been developed and might indicate an increasing incidence but in fact those pathogens could have been there already before. Also many of these pathogens often have no or mild symptoms and a high rate of spontaneous cure why the detection of the pathogen might actually be made *en passant*. Examples of this are rickettsioses, leptospirosis, *Coxiella burnetti* (Q-fever) and *Borrelia spp* [295-299].

There still needs to be a good collaboration between the clinician and laboratory for an accurate diagnosis to be made and to for example avoid over-reporting of positive results. Parola, Raoult et al that have contributed with new data from Africa on these types of infections. They also compared the presence of blood pathogens detected by PCR in febrile and afebrile children where malaria followed by *S. pneumoniae* was the dominant pathogens[274]. However, most of the mentioned emerging infections previously detected in adults were not found in children[274].

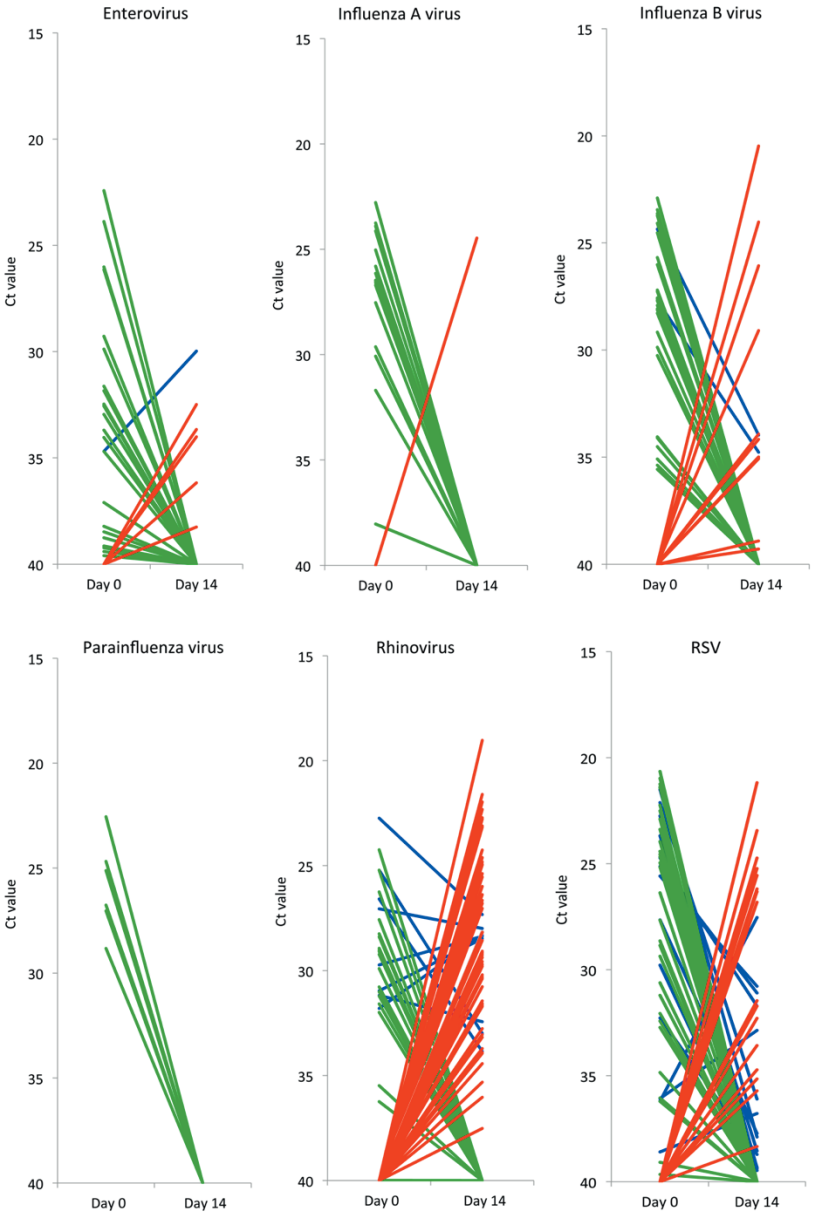


Figure 5. Real-time PCR Ct values on baseline (day 0) and follow-up (day 14) for selected pathogens. Each line represents one patient. Green lines for cleared infections and red lines for new infections on follow-up. Blue lines for agents detected at both baseline and follow-up. Ct values<40 were regarded negative.

Health outcome, pathogen clearance and new respiratory tract infections

Two children died out of seven of the included children that developed severe disease (study 2). Both of the deaths occurred five days after enrolment and none of them had a severe bacterial infection or abnormal laboratory values on inclusion. In 543/642 (85%) patients, the fever had resolved on both of the scheduled follow-ups. An additional 29 children had a verified fever relapse on the second follow-up visit (Figure 4 for fever resolution). On the 14-day follow-up, 207 patients were randomly selected for a pair-wise nasopharyngeal sampling and further real-time PCR analysis.

Ninety-five out of 207 (46%) had one or more new pathogens detected that were not seen on day 0. In 157/207 (76%) patients, 199 pathogens were detected in the nasopharynx on inclusion. Out of these infections 81% were not detected on the 14-day follow-up. More than 90% of infections with influenza A and B virus, enterovirus, and parainfluenza virus were cleared. RSV or rhinovirus constituted 72% of the 36 pathogens persisting on day 14, but also 44% (51/115) of new infections detected on day 14.

Considering fever resolution, out of the pair-sampled patients, 18% (37/207) were febrile on one or both of the follow-up days, of whom 18 patients had a new infection detected (influenza B (n=5), RSV (n=6), rhinovirus (n=5), metapneumovirus (n=1), enterovirus (n=1)). The high rates of influenza B and RSV infections on day 0 and among new infections on day 14 might reflect on-going epidemics with these viruses in Zanzibar at the time of the study. This would fit with the observation that influenza virus B often presented as new infections after inclusion, since it was the only pathogen significantly associated with presence of a measured fever on any follow-up time points (*paper IV*).

There was a high pathogen clearance rate for enteroviruses, influenza, and RSV (see Figure 5 for visualization) which is in line with previous

research that have shown an average shedding duration of less than 2 weeks[300-308]. Previous studies of the duration of respiratory infections have included fewer patients or have focused on one or a few pathogens, and report lower frequencies of pathogens detected[309].

Paper IV simultaneously analysed presence of 22 pathogens using a sensitive assay that allows for pathogen quantity estimation. Thereby, it was possible to detect reduced microbial load in addition to clearance of pathogens when comparing the first with the second sampling. This approach in combination with results from the sequencing to discriminate rhinovirus and enterovirus were in particular helpful when analysing rhinoviruses and comparing data from day 0 and day 14. Hereby, we could conclude that the high frequency of rhinovirus both on inclusion and follow-up was likely explained by many different rhinovirus types circulating simultaneously, resulting in frequent exposure.

New infections with rhinovirus (48/207; 23%) were also more common than persisting infections (12/207; 5.7%). These results are consistent with other longitudinal studies reporting that infections with new rhinovirus strains are more common than infections persisting >30 days[306 310 311]. Hereby, we were also able to identify that enterovirus, was cleared in essentially all cases.

Some previous aetiology studies disregarded the results from nasopharyngeal swab PCRs despite the fact that this is the established site of sampling for respiratory tract pathogens that cause a fever. The argument has mainly been that pathogens continue to be detected in the nasopharynx a long time after the acute illness and are therefore not significant. However, this could not be supported by our findings. Longitudinal sampling is crucial to understanding the course of respiratory tract infections in children.

The effects of seasonality

Study 2 was conducted during and just after the rain season and hereby, did not cover all tropical seasons which was a limitation of the study. The effect of seasonality on infectious diseases is important and depends on environmental factors (like temperature and rainfall), host factors (e.g. vector seasonality) and human behaviour (outdoor or indoor activities, school or summer holiday)[312]. In tropical and subtropical climates, these changes are in general connected to the annual rain or dry seasons. Typically, malaria incidence increases during or just after the rain season[313]. Influenza is present all year round although an increase in transmission during or just after the rain periods is pronounced[103 104], often coinciding with a decrease in temperature.

Similarly, RSV and general viral detection have also been shown to increase during and just after rain falls, in low-income countries in the tropical zones[306], but specific data from most parts of Africa is lacking[314 315]. For some pathogens, the epidemic pattern has to do with the dew point temperature (which is correlated to the level of humidity). For example enteroviruses that tend to peak during the warmer months, although this has mainly been shown in temperate rather than tropical climates[316].

Rotavirus transmission has also been described as rain or temperature dependent transmission but also the income level of the country where the patient resides seems to be important[317]. Thus, the low rate of rotavirus infections in our study might be due to the choice of time period for sampling, which also could have influenced the attributable fraction for some other pathogens. Also, the fact that we did not have any arboviral infections may be a seasonal effect since dengue and chikungunya epidemics tend to emerge during the hot[318] and dry season[279] rather than the rain season.

IMCI classifications versus final diagnosis

No previous reports have compared IMCI classifications with a final diagnosis based on comprehensive laboratory support and pre-defined study definitions. The few studies made are difficult to reproduce or verify and are neither comprehensive nor clinically useful[319] [123].

In our study, the most common IMCI classification was pneumonia, (n=387 (57%)), followed by common cold (n=224 (33%)), watery diarrhoea (n=164 (24%)), acute ear infection (16 (2%)), dysentery (n=12 (2%)), chronic ear infection (n=3 (0,4%)) and malaria (n=2 (0,3%)). The two patients with malaria also fulfilled the IMCI classification pneumonia. More than one IMCI classification was assigned to 25% of the patients and there was a substantial overlap between different IMCI classifications (Figure 6) what others also have described[143].

This complexity is illustrated by the finding that the symptoms in some cases were not typical of the final diagnoses (Figure 7), where for example RSV was the most commonly detected final diagnosis in patients with diarrhoea. Overlapping gastrointestinal and ARI symptoms have been described before in particular for coronaviruses[320] and bocavirus[321] . This brings to light that children have complex illnesses and many fulfil multiple IMCI classifications as well as final diagnoses[161]. This leads to the subsequent question, maybe even more important, who needs treatment, and who needs antibiotics.

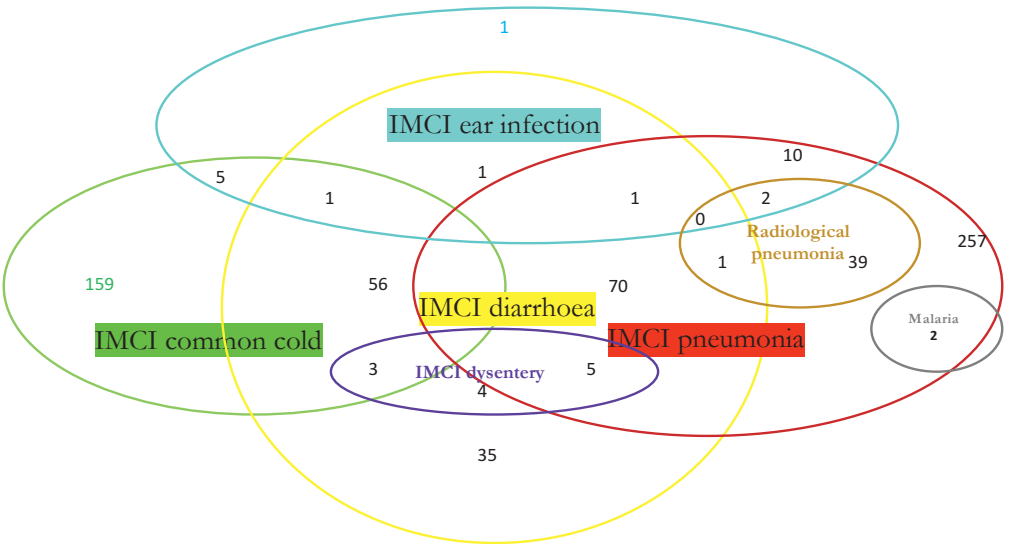


Figure 6. Venn diagram displaying overlapping IMCI diagnoses for IMCI pneumonia (n=387), IMCI common cold (n=224), IMCI diarrhoea (n=176), IMCI ear infection (n=21), and IMCI dysentery (n=12)

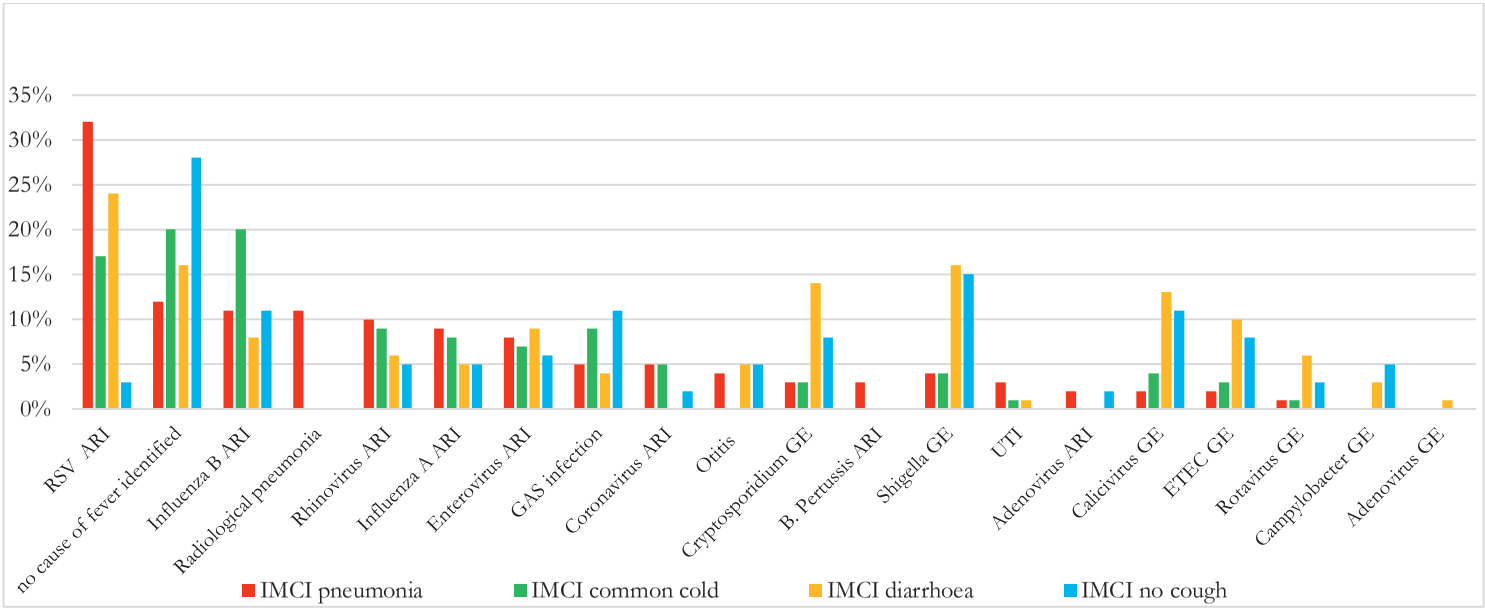


Figure 7. Causes of fever divided by IMCI classification. (IMCI no cough is defined as no IMCI common cold or no IMCI pneumonia) GE= gastroenteritis. ARI= acute respiratory tract infections

Infections that require antibiotics

A majority of the 5.8 million deaths in children is directly caused by infections that in many cases probably could be averted with antibiotics[322].

In study 2, 74% (500/677) of all patients received antibiotics, whereas the antibiotic prescription was lower in study 1 with 58% (1052/1822) to children below 5 years of age. All patients were managed according to the IMCI, and the health workers were trained in a similar way for both studies. Almost every fourth patient (152/677, 22%) had a bacterial infection presumed to require antibiotics (Figure 8). The most common were throat and/or skin infection with *S. pyogenes* (GAS) (6%), x-ray verified pneumonia (6%), *Shigella* gastroenteritis (4%), otitis media (4%) and urinary tract infection (2%).

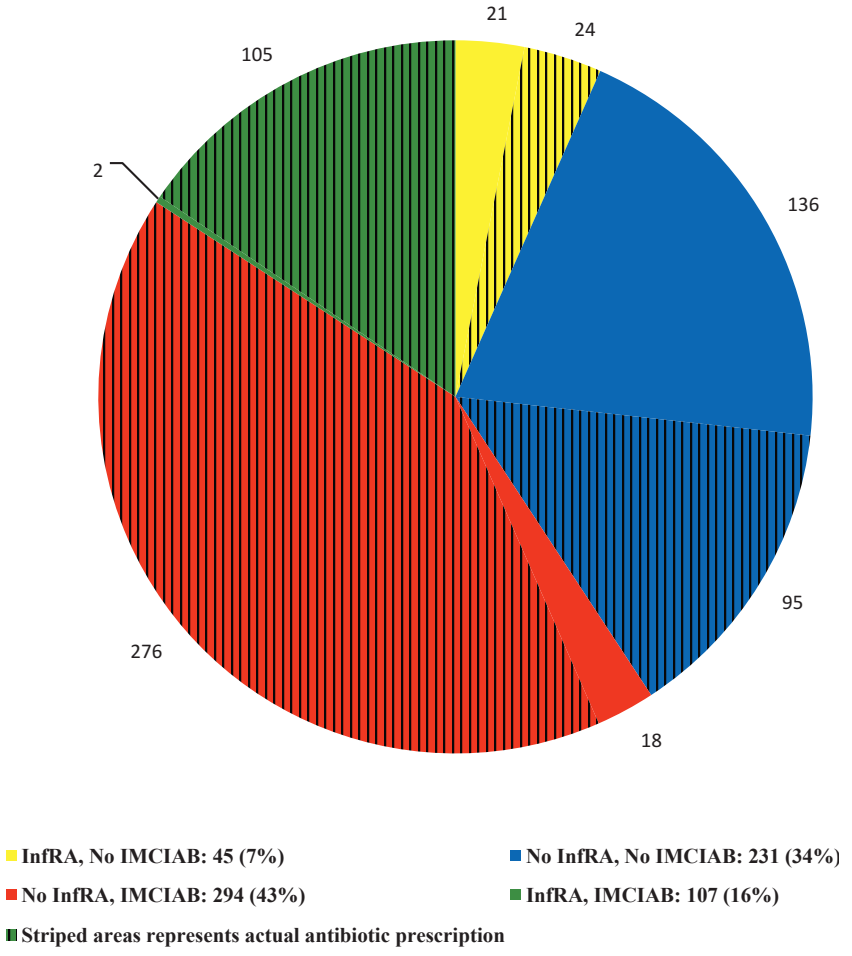


Figure 8. Proportions of patients with an antibiotic indication by IMCI (IMCIAB) and actual antibiotic prescription in patients with and without an infection requiring antibiotics (InfRA/no InfRA). Striped areas represent actual antibiotic prescription

Shigella infections

Shigellosis is after rotavirus the second leading cause of diarrhoeal mortality globally with 60 000 deaths among children below 5 years old[6]. Both of the pathogens were associated with fever in the MAL-ED longitudinal birth cohort study on enteropathogen contribution to diarrhoeal disease[12].

In study 2, 4% of all patients had *Shigella* infection as final diagnosis and 16% of those with diarrhoea or dysentery. Antibiotic treatment is recommended for shigellosis infection[323]. However, the most recent Cochrane report compared antibiotics to placebo and concluded that a patient that has a mild diarrhoea might not need treatment even if Shigella is detected in the stool. In contrast to this, a recent large meta-analysis showed that the presence of shigellosis is associated with a mortality rather than dysentery, [324] . Hence, effective ways to find those children that need antibiotics must be investigated[186 324].

Half of our patients with *Shigella* infections were supposed to receive an antibiotic according to the IMCI. Yet, as seen in the Venn diagram in Figure 6, only a few would receive the treatment for the indication *Shigella* dysentery but instead most for a pneumonia which is treated with Amoxicillin. This is nowadays not sufficient to treat shigellosis, instead either a fluoroquinolone or a cephalosporin is recommended. However, *Shigella* strains have emerged that are resistant to these antibiotics, but these strains are not so common in Africa as in Asia[325].

Group A streptococci

Group A streptococci (GAS) are one of the most common and also dangerous causes of morbidity and mortality in children. The highest disease burden is in resource limited settings where complications, such as septicaemia, acute rheumatic fever, rheumatic heart disease, and acute post-streptococcal glomerulonephritis seem to be more frequent. Therefore, in these settings, GAS tonsillitis should be treated also for

prevention of complications. In recent years, the usefulness of the Centor criteria for clinical diagnosis of GAS infection has been questioned, both to rule in as well as rule out the infection[326].

Immunochromatographic tests that detect GAS from throat swabs are therefore widely used in conjunction with the Centor criteria, in high-income settings but not as much in LMICs. Our study had a high rate of crude GAS detection of 14% (QuickVue®), and still after the application of the pre-defined study criteria that considered reported symptoms and concomitantly detected pathogens, the rate was 6%. In contrast, Kiemde et al performed a similar study in Kenya where 10% of included patients were treated with antibiotics prior to inclusion (similar to *paper II* with 8%). They included febrile patients and healthy controls 1-12 years old presenting to a hospital outpatient department. Of these, only 2.3% of patients and 0.6% of afebrile subjects were positive for GAS by rapid test (Clearview®) and/or culture on throat swabs[249].

Interpretation of GAS rapid diagnostic tests might be a source of both false positive and false negative results. Most immunochromatographic tests like Strep A tests and mRDTs define positivity regardless of band intensity. This could be one reason why D'Acremont et al had no patients positive for GAS on throat swabs, but more likely it was due to a narrow criteria (history of sore throat) for rapid test sampling since it is difficult to assess throat pain in a child <5 years old[161].

Urinary tract infections

Urinary tract infections (UTI) and pyelonephritis are one of the most common bacterial causes of fever in children, especially in infants with the long term risk of renal damage. Most studies find rather unspecific symptomatology in patients with UTI[327]. UTI prevalence is estimated to be more than 10% in admitted febrile children <5 years of age in SSA[327-329]. Our study showed that 2% of febrile young children had a UTI, but the crude prevalence in this population is probably higher if children without fever and those few with concomitant UTI and diarrhoea would be sampled. In addition, the study definition of a UTI was; a significant

growth in urine of $\geq 10^7$ colony forming units/L as well as a positive leukocyte esterase or nitrite in urine. Indeed, other studies disregarded the latter of the mentioned criteria and included more symptoms to find the UTIs although this might lead to a risk of interpreting asymptomatic bacteriuria that does not need a treatment as a UTI that require antibiotics[330].

Many children in low resource settings do not have access to prescription of the right antibiotic in due time when they need it. Indeed, *paper II* showed that not only over- but also under-prescription or -treatment of antibiotics is a fact in low- and middle-income countries like Zanzibar[331] [332].

To serve as an example, a Nadjm et al showed that one third of children admitted due to febrile illness that needed antibiotics still did not receive it when assessed and managed according to the WHO guideline *"Management of the child with a serious infection or severe malnutrition: guidelines for care at the first-referral level in developing countries"*. A high rate of antibiotic resistance was also seen. Also, half of the patients with an SBI had a non-typhoidal salmonellosis which probably would not be cured by the prescribed antibiotic treatment anyway[333] due to antibiotic resistance of NTS and/or that most first or even second line antibiotics do not target NTS.

IMCI antibiotic treatment versus antibiotic requirement

Among the 525 patients without infections requiring antibiotics, 294 (56%) had an IMCI indication for antibiotics, a majority of them due to IMCI pneumonia (*paper II*). The two patients with malaria in study 2 received antibiotics because of IMCI pneumonia (Figure 6), which was the most common cause of "overtreatment" in study 2. In study 1, the antibiotic prescription rate was significantly higher in older children and adults in RDT negative (1145/1968 (58%)) than RDT positive patients (14/85 (16%)). However, in children under five it was similar in RDT positive and RDT negative patients of whom most received an antibiotic due to IMCI pneumonia.

These results are in line with a study from Zanzibar by Msellem et al that reported an antibiotic prescription of 69% in children under 5[20]. Moreover, they showed significantly higher antibiotic prescription in patients tested with mRDT and in those with clinical presumptive diagnosis[20 334]. The latter study was performed in 2005 when every third patient had malaria but a recent meta-analysis of over 500 000 consultations in Africa and Asia confirms this effect of mRDTs on antibiotic prescription. Antibiotic prescription was consistently and significantly higher through all studies in sites that used an mRDT. It is important not to draw conclusions too quickly since overprescription was probably there already before, but might have been brought to light by these recent studies.

Yet, a multi-country evaluation that analysed demographic health survey data reported clear differences between countries after the introduction of mRDTs. Some countries had a significantly higher and some lower antibiotic and/or antimalarial prescription rate in patients that were tested with an mRDT [335]. This shows the complexity of introduction of a new diagnostic test and the need for supervision and surveillance.

Like study 2, other comprehensive studies have also shown that most children have a viral infection and only a minority of the children with fever need antimicrobial treatment[161 162 249 264]. Health workers need support in further fever management of negative mRDT patients including guidance on antibiotic and antimalarial prescription[336 337].

One successful example is the e-health algorithm ALMANACH, which was developed based on the results from d'Acremont et al[161] and is a further development of an electronic version of the IMCI guideline. The most important revision is the change in the cut-off that define a fast breathing from 40 breaths/minute in IMCI (2014) to 50 breaths /minute in ALMANACH and the addition of a urine dipstick and a typhoid test to selected patients[338]. The ALMANACH approach was evaluated in a non-inferiority trial in Tanzania and there was no difference in health outcome but antibiotic prescription decreased with 80%[339].

Ameyaw et al combined the introduction of a malaria RDT with a test and treat package in the outpatient department of a hospital in Ghana. Patients that had a normal clinical examination - no positive findings on examination of the lungs, ear, throat and joints - and a negative malaria test and urine dipstick for leukocytes and nitrites were regarded to have a "possible viral infection"[340]. Moreover, for those that were diagnosed with a bacterial infection, the choice of antibiotic was amoxicillin-clavulanic acid to cover for urinary tract infections. They showed that patients of the test-treat arm were prescribed less antimalarials and antibiotics and still had a significantly better outcome (less hospitalizations) than routine care.

Is pneumonia the new malaria?

Bacterial infections are the most common cause of mortality in children globally. Hence, many patients are in need of antibiotics, but overtreatment is common. As already mentioned, after the introduction of malaria RDTs there is a worry that patients with a negative malaria test would automatically receive antibiotics instead[341]. This is further demonstrated by the case of pneumonia. *Paper II* showed that 57% of patients had an IMCI pneumonia of whom 42/342 (12%) had a radiological pneumonia. This result is in line with previous studies that have a chest X-ray verification in 10-20% of patients with clinically suspected pneumonia[144 342-344] of which most but not all used the systematic WHO criteria used in *paper II*[148].

But should all antibiotic courses given to children that did not have a positive chest X-ray really be classified as overtreatment? Critics to using CXR as gold standard claim that the presence of an infiltrate is independent of both final outcome and aetiology[140]. In addition, qualitative radiology services are rare in resource-limited settings, and the main clinical reason against is that there is some delay until a pneumonia is visible on the radiograph[345]. Nevertheless, in the PERCH study there was an increased risk of death within a 30-day follow-up period for children with an abnormal CXR[346]. Also, the disease reductions after the introduction of pneumococcal vaccines were seen for invasive pneumococcal disease and radiological pneumonia but not for IMCI pneumonia[347].

Patients with IMCI pneumonia might have other causes of disease like bacteraemia or otitis media that are averted with the antibiotic treatment intended for the pneumonia. This is illustrated by a study from Tanzania in 2005. Before the introduction of pneumococcal vaccine almost 7% of patients with mild pneumonia had sepsis, 38% of which was due to invasive pneumococcal disease[348].

What happens with febrile patients that do not receive any antibiotic or antimalarial treatment? In a study from Zambia, most RDT negative

children recovered on antipyretics alone[349]. In study 2, the prevalence of severe bacterial infections (SBI) was 50/677 (7,4%). Three of these patients did not receive antibiotics but recovered anyway (*paper II*). A proportion of infections that according to guidelines require antibiotics do heal without treatment, for example otitis media, pneumonia[350], GAS tonsillitis and *Shigella* infections.

So out of all patients with IMCI pneumonia, who benefits from antibiotic treatment? In the last decade the duration of antibiotic treatment for IMCI pneumonia (2014 version) has been reduced from 5 days to optional 3 days based on studies in Asia[351]. Furthermore, studies from Papua Guinea and Pakistan reported no differences in outcome in non-severe IMCI/WHO pneumonias with or without first-line antibiotic treatment[352 353]. Also, a 2016 Cochrane report concluded that there was not sufficient evidence for the benefit of antibiotics to neither otitis nor fast breathing pneumonia according to IMCI[350] and that there was a need for further evidence.

Thus, recently a randomized control study from Malawi again evaluated the 3-day amoxicillin regimen versus placebo in children 2-59 months with non-severe IMCI pneumonia. There were no deaths, but the study was prematurely interrupted due to a higher rate of treatment failures in the placebo group. The number-needed-to-treat to prevent one failure was 33 patients.

Further analysis of the clinical data in the Malawi study did not find any prognostic risk score to aid antibiotic decision-making[354]. A retrospective sub-group analysis was conducted of community recruited children in Malawi who did not receive antibiotic treatment (cotrimoxazole) for IMCI pneumonia. Patients without treatment had worse clinical outcomes than patients that were treated with antibiotics. They were also more likely to be sicker on inclusion. Again the need for clinical decision support is vital, including which treatment to recommend for pneumonia. In particular in a high endemic HIV setting with a probable higher proportion of fevers that are caused by gram negative infections, cotrimoxazole might be better than amoxicillin [355].

Inflammatory biomarkers to guide management

Most of the management guidelines for preschool children used on the first level of health care in resource limited settings, combine history, symptoms and vital signs to distinguish a healthy from a sick child [21]. However, using only clinical examination has an unsatisfactory level of sensitivity and specificity for detection of severe disease.

Could inflammatory biomarkers like C-reactive Protein (CRP) or procalcitonin (PCT) be a tool both to rule in or rule out infection? CRP is an acute-phase reactant used in primary health care world-wide to distinguish viral from bacterial disease and has been shown to be helpful in the identification of which children that have serious bacterial infections in Europe[218] and Africa[345 346]. CRP has not been evaluated as a complement to IMCI in neither pneumonia diagnosis nor overall antibiotic requirement, mostly because comprehensive aetiology studies on all-cause febrile illness have not been performed.

In study 2, CRP was the only independent variable significantly associated with radiological pneumonia in a univariate and multiple logistic regression ($p<0.001$), but a cut-off for identification of CXR verified pneumonia that had an acceptable level of sensitivity could not be found. For example, 36% of patients with CXR pneumonia had a CRP <20 mg/dL. The PERCH study showed in line with study 2 that CRP was significantly higher in those patients with a confirmed bacterial pneumonia than with an RSV pneumonia[356]. ROC-analysis showed that a cut-off of 37.1 mg/L had the best discrimination. Still, the sensitivity of this cut-off was 77% and it might not be acceptable not to treat 23% of the pneumonias that are likely to need treatment.

Keitel et al further developed the E-POCT guideline based on the above mentioned ALMANACH guideline and added both CRP and PCT to selected patients. A non-inferiority trial showed that the E-POCT caused a reduction in antibiotic prescription from 95% to 11.5% as well as a significant reduction in clinical failures[357]. Similar results have emerged from other sites in Asia[358 359].

In the last decade, there has been an increasing interest in various biomarkers for use in the clinical decision of antibiotic treatment. CRP has stood out as the inflammatory marker candidate that has the potential to reduce antibiotic prescription with cut-offs for treatment above 10-40 mg/L[356 359-363] ,. There are also available low cost POC tests[364]. There might be a possible development of stepwise decision models using so called classification and regression tree analysis (CART)[102 365]. The million-dollar question however, is to find the patient that *needs* antibiotics in routine clinical care. This should be given higher priority than finding those that do *not need* it. So in the meantime, there can be no other choice for the frontline health care workers in low resource settings but to choose the antibiotic rather than no treatment.

CONCLUSIONS

- The sensitivity of the malaria rapid diagnostic tests in comparison with both malaria microscopy as well as malaria PCR was relatively low. Thus, in addition to quality control and supervision more sensitive tools than histidine-rich protein 2 (HRP-2) based mRDTs might be called for in a malaria pre-elimination setting like Zanzibar.
- The adherence of health workers to the mRDT was excellent also when used as a part of the IMCI. This shows that the mRDT can reliably be integrated into the IMCI for malaria diagnosis.
- The vast majority of children with uncomplicated fevers presenting to primary health care have a viral infection as a cause of fever. One in four has an infection that requires antibiotics.
- It is common that fever patients and healthy controls have similar detection rates of respiratory tract pathogens and diarrhoeal pathogens.
- The interpretation of threshold cycle (Ct) values as a proxy for pathogen load in faecal samples may, for some pathogens, be crucial when attributing cause of symptoms.
- Children with acute uncomplicated febrile illness in Zanzibar had a generally favourable outcome and rapidly cleared respiratory tract infections, but they frequently acquired new infections within two weeks.
- The current fever management guideline, IMCI, does not seem to adequately identify neither children that need antibiotics, nor those that do not.
- C-reactive Protein might be a useful biomarker for future intervention studies in particular to rule out that a patient has an antibiotic requiring infection.

PERSONAL REFLECTIONS AND FUTURE PERSPECTIVES

Twenty years ago I came as a tourist by boat from mainland Tanzania to Zanzibar. The reaction that I got before going there from people living in Dar Es Salaam was, "Oh Zanzibar, it is beautiful but beware of all the malaria".

Since then, malaria community prevalence has decreased by 96%[19] and tourists are now being advised not to take malaria prophylaxis due to the low malaria risk. Health care workers are aware of the fact that malaria is not as common anymore[366] and in 2009-2010, during the planning and conduct of the first study in this thesis we saw that the health care workers trusted the mRDTs and managed patients according to it[336]. Yet, the question that often came up was; "Ok, if it is not malaria, then what is it?". This thesis also aimed at answering that question.

By providing extended explorative information on aetiology and comparing it with clinical signs and symptoms, this project added knowledge for improved management of the feverish child through accurate diagnosis and ultimately better treatment.

The main results that emerged were that the rapid diagnostic tests for malaria used in Zanzibar in 2010 were below standards and that non-severely ill febrile children below the age of five in Zanzibar had viral infections. So called tropical illnesses were rare. Comparison with healthy controls provided invaluable information on aetiology. Both over- and underprescription of antibiotics was common but no clinical sign or laboratory test could single out antibiotic requirement in a child. By comparing the present results with recent studies from other research groups this thesis also outlined typical examples of common risks of bias and pitfalls that could be encountered in clinical research on infectious disease aetiologies.

Improved diagnosis of febrile illnesses in children in areas that lack access to health care with qualified staff and modern equipment must be a top priority. Zanzibar could serve as a model for the rest of Africa making this study a pilot project not only for East Africa but the whole SSA, particularly for low endemic malaria regions. With the results, we aimed to shed light on febrile disease in low-resource settings and provide hands-on paediatric health information for local, regional and global decision-makers.

Following the recent decades of large success in malaria control, there are worrying indications that the reduction has halted in the region[367] in the last couple of years, as indeed foreseen by many researchers and policy makers[19].

The WHO Malaria report issued in 2018[26] a warning that on a global level, no significant progress in reducing malaria cases was made in the recent couple of years. Instead malaria even increased in the ten countries with the highest malaria burden, with an estimated 3.5 million more cases of malaria in 2017 in comparison with 2016[368]. The ambitious target of the Sustainable Development Goals (SDG) initiative to reduce malaria incidence and prevalence with 90% from 2015 to 2030 is threatened. Only 50% of people living in malaria endemic countries have access to vector control and only 20% of pregnant women receive intermittent presumptive treatment of malaria. This is alarming and should be the focus of policy maker attention. But non-malarial fevers must still not be forgotten since mortality in this group is often higher.

What implications might the results of the study have on routine clinical management, on Research and Development or on advocacy issues in Zanzibar and sub-Saharan Africa? What is the way forward? Firstly, for future studies to be useful, a less exploratory approach should be sought by using well defined and reproducible qualitative or quantitative methods that aimed to improve important clinical outcomes, like reduced mortality or hospitalisation and improved epidemiology and outbreak surveillance, so called "Translational research".

Secondly, with this in mind, is the introduction of additional POC tests to the already widely implemented malaria RDTs the future of paediatric fever case management in Africa? Or should management instead include key equity predictors in the clinical assessment to receiving the right treatment like level of maternal education, country/place of residence, and socio-economic status[369]?

It is already now evident in Africa, that a high percentage of children do not receive testing or treatment for malaria or pneumonia[370]. Is adding on to the number of clinical assessments and procedures that the health care worker has to perform on the patients going to save lives or alleviate suffering? If one has to prioritize, maybe a focus on decreasing the amount of time it takes to get to proper health care or investing in emergency triage and treatment in the peripheral health facilities would have more impact on morbidity and mortality.

POC tests started to be of interest after the relative successful introduction of malaria rapid diagnostic tests. A POC test should be easy to interpret, and preferably provide a qualitative test result, i.e. positive/negative. It should directly give information on treatment or management, take less than 15 minutes, be cheap or at least have the potential of being low cost, preferably not having a need for cold chain or electricity. The WHO have set up the following mnemonic for the ideal POC tests: ASSURED (Affordable, Sensitive, Specific, User friendly, Rapid (treatment at first visit) and Robust (no cold chain needed), Equipment free and Delivered to those who need it)[371].

As much as I believe in the importance of providing up-dated regional data on infectious diseases in poor countries, I see a possible danger with the implementation of new and appealing techniques like metagenomics or a variety of POC tests in that it could satisfy the interests of academics, politicians, military researchers or philanthropists in microbes or global vital statistics data but it might not be important for survival of the poorest. Just because something is common does not mean that there is a need for a POC test in daily routine care. For example, a POC test for dengue case management in Asia probably does not save lives or improve

clinical management to the extent as an RDT for malaria in Africa does. On the other hand, it may be very useful for surveillance and case detection[371]. However, a POC test for the decision on when not to treat with antibiotics might provide a safer option than placebo for the treatment of clinical pneumonias in the peripheral health facilities.

Also, with the higher set SDGs that have replaced the Millennium Development Goals (MDGs) which should reach every child on the planet, a POC test may be advocated for. In my view, whatever the intervention, the most important thing is to remember, that the time is over when we can believe that a few diagnostic tests, guidelines, vaccines or antibiotics save all.

The future to improve the outcome of children with fever in Africa is to apply equity standards, and focus more on the poorest and youngest. It is time to get rid of poor clinical management of poor children living in poor areas. New promising techniques be it telemedicine[372], electronic management tools[373], metabolomics[374], or metagenomics[375] still need accurate clinical management, laboratory standards of procedures and data provision on ground. There is still the need of a health worker or laboratory assistant to correctly prepare a blood slide, conduct an X-ray or a point-of-care ultrasound. Thus, investing in human resources is probably even more important under those circumstances. Another test needs just as much human involvement and quality assurance, only in another way. And remember, poverty is still the main killer in children and not pathogens.

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REFERENCES

1. You D, Hug L, Ejdemyr S, et al. Global, regional, and national levels and trends in under-5 mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality Estimation. Lancet 2015;**386**(10010):2275-86.
2. Wang H, Liddell CA, Coates MM, et al. Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;**384**(9947):957-79.
3. Feikin DR, Olack B, Bigogo GM, et al. The burden of common infectious disease syndromes at the clinic and household level from population-based surveillance in rural and urban Kenya. PLoS One 2011;**6**(1):e16085.
4. Ansah EK, Gyapong M, Narh-Bana S, et al. Factors influencing choice of care-seeking for acute fever comparing private chemical shops with health centres and hospitals in Ghana: a study using case-control methodology. Malar J 2016;**15**(1):290.
5. Global Burden of Disease C, Adolescent Health C, Kassebaum N, et al. Child and Adolescent Health From 1990 to 2015: Findings From the Global Burden of Diseases, Injuries, and Risk Factors 2015 Study. JAMA Pediatr 2017;**171**(6):573-92.
6. Collaborators GBDDD. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 2018;**18**(11):1211-28.
7. Collaborators GL. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017;**17**(11):1133-61.
8. Kiemde F, Spijker R, Mens PF, et al. Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Trop Med Int Health 2016;**21**(8):943-55.
9. Prasad N, Murdoch DR, Reyburn H, et al. Etiology of Severe Febrile Illness in Low- and Middle-Income Countries: A Systematic Review. PLoS One 2015;**10**(6):e0127962.
10. Crump JA. Time for a comprehensive approach to the syndrome of fever in the tropics. Trans R Soc Trop Med Hyg 2014;**108**(2):61-2.
11. Crump JA, Kirk MD. Estimating the Burden of Febrile Illnesses. PLoS Negl Trop Dis 2015;**9**(12):e0004040.
12. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health 2015;**3**(9):e564-75.
13. Pneumonia Etiology Research for Child Health Study G. Causes of severe pneumonia requiring hospital admission in children without HIV infection

from Africa and Asia: the PERCH multi-country case-control study. Lancet 2019.
14. Levine OS, O'Brien KL, Deloria-Knoll M, et al. The Pneumonia Etiology Research for Child Health Project: a 21st century childhood pneumonia etiology study. Clin Infect Dis 2012;**54 Suppl 2**:S93-101.
15. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 2013.
16. Benet T, Sanchez Picot V, Messaoudi M, et al. Microorganisms Associated With Pneumonia in Children <5 Years of Age in Developing and Emerging Countries: The GABRIEL Pneumonia Multicenter, Prospective, Case-Control Study. Clin Infect Dis 2017.
17. Hercik C, Cosmas L, Mogeni OD, et al. A Combined Syndromic Approach to Examine Viral, Bacterial, and Parasitic Agents among Febrile Patients: A Pilot Study in Kilombero, Tanzania. Am J Trop Med Hyg 2018;**98**(2):625-32.
18. Waitumbi JN, Kuypers J, Anyona SB, et al. Outpatient upper respiratory tract viral infections in children with malaria symptoms in Western Kenya. Am J Trop Med Hyg 2010;**83**(5):1010-3.
19. Bjorkman A, Shakely D, Ali AS, et al. From high to low malaria transmission in Zanzibar-challenges and opportunities to achieve elimination. BMC Med 2019;**17**(1):14.
20. Msellem MI, Martensson A, Rotllant G, et al. Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar: a crossover validation study. PLoS Med 2009;**6**(4):e1000070.
21. English M, Scott JA. What is the future for global case management guidelines for common childhood diseases? PLoS Med 2008;**5**(12):e241.
22. Van Hemelrijck MJ, Lindblade KA, Kubaje A, et al. Trends observed during a decade of paediatric sick visits to peripheral health facilities in rural western Kenya, 1997-2006. Trop Med Int Health 2009;**14**(1):62-9.
23. Prasad N, Sharples KJ, Murdoch DR, et al. Community prevalence of fever and relationship with malaria among infants and children in low-resource areas. Am J Trop Med Hyg 2015;**93**(1):178-80.
24. Elmardi KA, Noor AM, Githinji S, et al. Self-reported fever, treatment actions and malaria infection prevalence in the northern states of Sudan. Malar J 2011;**10**:128.
25. Novignon J, Nonvignon J. Socioeconomic status and the prevalence of fever in children under age five: evidence from four sub-Saharan African countries. BMC Res Notes 2012;**5**:380.
26. WHO. World Malaria Report 2018. 2018.
27. Diene Sarr F, Niang M, Thiam D, et al. Acute Febrile Illness and Influenza Disease Burden in a Rural Cohort Dedicated to Malaria in Senegal, 2012-2013. PLoS One 2015;**10**(12):e0143999.

28. Yaya S, Bishwajit G. Burden of Acute Respiratory Infections Among Under-Five Children in Relation to Household Wealth and Socioeconomic Status in Bangladesh. Trop Med Infect Dis 2019;**4**(1).
29. Alene M, Yismaw L, Berelie Y, et al. Health care utilization for common childhood illnesses in rural parts of Ethiopia: evidence from the 2016 Ethiopian demographic and health survey. BMC Public Health 2019;**19**(1):57.
30. Castellani J, Mihaylova B, Evers SM, et al. Out-of-pocket costs and other determinants of access to healthcare for children with febrile illnesses: a case-control study in rural Tanzania. PLoS One 2015;**10**(4):e0122386.
31. Global Burden of Disease Health Financing Collaborator N. Past, present, and future of global health financing: a review of development assistance, government, out-of-pocket, and other private spending on health for 195 countries, 1995-2050. Lancet 2019.
32. Ozawa S, Clark S, Portnoy A, et al. Estimated economic impact of vaccinations in 73 low- and middle-income countries, 2001-2020. Bull World Health Organ 2017;**95**(9):629-38.
33. Shretta R, Avancena AL, Hatefi A. The economics of malaria control and elimination: a systematic review. Malar J 2016;**15**(1):593.
34. Escribano Ferrer B, Hansen KS, Gyapong M, et al. Cost-effectiveness analysis of the national implementation of integrated community case management and community-based health planning and services in Ghana for the treatment of malaria, diarrhoea and pneumonia. Malar J 2017;**16**(1):277.
35. Watkins D, Lubinga SJ, Mayosi B, et al. A Cost-Effectiveness Tool to Guide the Prioritization of Interventions for Rheumatic Fever and Rheumatic Heart Disease Control in African Nations. PLoS Negl Trop Dis 2016;**10**(8):e0004860.
36. Chow A, Robinson JL. Fever of unknown origin in children: a systematic review. World J Pediatr 2011;**7**(1):5-10.
37. Jiang L, Lee VJ, Lim WY, et al. Performance of case definitions for influenza surveillance. Euro Surveill 2015;**20**(22):21145.
38. Lutterloh E, Likaka A, Sejvar J, et al. Multidrug-resistant typhoid fever with neurologic findings on the Malawi-Mozambique border. Clin Infect Dis 2012;**54**(8):1100-6.
39. Svenson JE, MacLean JD, Gyorkos TW, et al. Imported malaria. Clinical presentation and examination of symptomatic travelers. Arch Intern Med 1995;**155**(8):861-8.
40. Kananura RM, Kiwanuka SN, Ekirapa-Kiracho E, et al. Persisting demand and supply gap for maternal and newborn care in eastern Uganda: a mixed-method cross-sectional study. Reprod Health 2017;**14**(1):136.
41. Steinhardt LC, Chinkhumba J, Wolkon A, et al. Quality of malaria case management in Malawi: results from a nationally representative health facility survey. PLoS One 2014;**9**(2):e89050.

42. Maheu-Giroux M, Casapia M, Gyorkos TW. On the validity of self-reports and indirect reports to ascertain malaria prevalence in settings of hypoendemicity. Soc Sci Med 2011;**72**(5):635-40.
43. Dunyo SK, Koram KA, Nkrumah FK. Fever in Africa and WHO recommendation. Lancet 1997;**350**(9090):1550.
44. Einterz EM, Bates ME. Fever in Africa: do patients know when they are hot? Lancet 1997;**350**(9080):781.
45. Akinbami FO, Orimadegun AE, Tongo OO, et al. Detection of fever in children emergency care: comparisons of tactile and rectal temperatures in Nigerian children. BMC Res Notes 2010;**3**:108.
46. Li YW, Zhou LS, Li X. Accuracy of Tactile Assessment of Fever in Children by Caregivers: A Systematic Review and Meta-analysis. Indian Pediatr 2017;**54**(3):215-21.
47. Okiro EA, Snow RW. The relationship between reported fever and Plasmodium falciparum infection in African children. Malar J 2010;**9**:99.
48. Mutanda AL, Cheruiyot P, Hodges JS, et al. Sensitivity of fever for diagnosis of clinical malaria in a Kenyan area of unstable, low malaria transmission. Malar J 2014;**13**:163.
49. Chandramohan D, Carneiro I, Kavishwar A, et al. A clinical algorithm for the diagnosis of malaria: results of an evaluation in an area of low endemicity. Trop Med Int Health 2001;**6**(7):505-10.
50. Pigott DM, Howes RE, Wiebe A, et al. Prioritising Infectious Disease Mapping. PLoS Negl Trop Dis 2015;**9**(6):e0003756.
51. Gunalan K, Niangaly A, Thera MA, et al. Plasmodium vivax Infections of Duffy-Negative Erythrocytes: Historically Undetected or a Recent Adaptation? Trends Parasitol 2018;**34**(5):420-29.
52. Howes RE, Patil AP, Piel FB, et al. The global distribution of the Duffy blood group. Nat Commun 2011;**2**:266.
53. Baltzell KA, Shakely D, Hsiang M, et al. Prevalence of PCR detectable malaria infection among febrile patients with a negative Plasmodium falciparum specific rapid diagnostic test in Zanzibar. Am J Trop Med Hyg 2013;**88**(2):289-91.
54. Gething PW, Elyazar IR, Moyes CL, et al. A long neglected world malaria map: Plasmodium vivax endemicity in 2010. PLoS Negl Trop Dis 2012;**6**(9):e1814.
55. Albsheer MMA, Pestana K, Ahmed S, et al. Distribution of Duffy Phenotypes among Plasmodium vivax Infections in Sudan. Genes (Basel) 2019;**10**(6).
56. O'Meara WP, Mangeni JN, Steketee R, et al. Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect Dis 2010;**10**(8):545-55.
57. Maltha J, Guiraud I, Lompo P, et al. Accuracy of PfHRP2 versus Pf-pLDH antigen detection by malaria rapid diagnostic tests in hospitalized children in a seasonal hyperendemic malaria transmission area in Burkina Faso. Malar J 2014;**13**:20.

58. Kiemde F, Tahita MC, Lompo P, et al. Treatable causes of fever among children under five years in a seasonal malaria transmission area in Burkina Faso. Infect Dis Poverty 2018;**7**(1):60.
59. Kiggundu VL, O'Meara WP, Musoke R, et al. High prevalence of malaria parasitemia and anemia among hospitalized children in Rakai, Uganda. PLoS One 2013;**8**(12):e82455.
60. Sypniewska P, Duda JF, Locatelli I, et al. Clinical and laboratory predictors of death in African children with features of severe malaria: a systematic review and meta-analysis. BMC Med 2017;**15**(1):147.
61. Mathison BA, Pritt BS. Update on Malaria Diagnostics and Test Utilization. J Clin Microbiol 2017;**55**(7):2009-17.
62. Veiga MI, Ferreira PE, Malmberg M, et al. pfmdr1 amplification is related to increased Plasmodium falciparum in vitro sensitivity to the bisquinoline piperaquine. Antimicrob Agents Chemother 2012;**56**(7):3615-9.
63. Kwenti TE, Njunda LA, Tsamul B, et al. Comparative evaluation of a rapid diagnostic test, an antibody ELISA, and a pLDH ELISA in detecting asymptomatic malaria parasitaemia in blood donors in Buea, Cameroon. Infect Dis Poverty 2017;**6**(1):103.
64. Yman V, White MT, Rono J, et al. Antibody acquisition models: A new tool for serological surveillance of malaria transmission intensity. Sci Rep 2016;**6**:19472.
65. Tarimo DS, Minjas JN, Bygbjerg IC. Malaria diagnosis and treatment under the strategy of the integrated management of childhood illness (IMCI): relevance of laboratory support from the rapid immunochromatographic tests of ICT Malaria P.f/P.v and OptiMal. Ann Trop Med Parasitol 2001;**95**(5):437-44.
66. Reyburn H. New WHO guidelines for the treatment of malaria. BMJ 2010;**340**:c2637.
67. Abba K, Deeks JJ, Olliaro P, et al. Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries. Cochrane Database Syst Rev 2011(7):CD008122.
68. Mtove G, Hendriksen IC, Amos B, et al. Treatment guided by rapid diagnostic tests for malaria in Tanzanian children: safety and alternative bacterial diagnoses. Malar J 2011;**10**:290.
69. d'Acremont V, Malila A, Swai N, et al. Withholding antimalarials in febrile children who have a negative result for a rapid diagnostic test. Clin Infect Dis 2010;**51**(5):506-11.
70. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 2009;**361**(5):455-67.
71. Baiden F, Malm K, Bart-Plange C, et al. Shifting from presumptive to test-based management of malaria - technical basis and implications for malaria control in Ghana. Ghana Med J 2014;**48**(2):112-22.
72. Abba K, Kirkham AJ, Olliaro PL, et al. Rapid diagnostic tests for diagnosing uncomplicated non-falciparum or Plasmodium vivax malaria in endemic countries. Cochrane Database Syst Rev 2014(12):CD011431.

73. Okell LC, Bousema T, Griffin JT, et al. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nature communications 2012;**3**:1237.
74. Manjurano A, Okell L, Lukindo T, et al. Association of sub-microscopic malaria parasite carriage with transmission intensity in north-eastern Tanzania. Malaria journal 2011;**10**:370.
75. Ngasala BE, Malmberg M, Carlsson AM, et al. Efficacy and effectiveness of artemether-lumefantrine after initial and repeated treatment in children <5 years of age with acute uncomplicated Plasmodium falciparum malaria in rural Tanzania: a randomized trial. Clin Infect Dis 2011;**52**(7):873-82.
76. Malmberg M, Ferreira PE, Tarning J, et al. Plasmodium falciparum drug resistance phenotype as assessed by patient antimalarial drug levels and its association with pfmdr1 polymorphisms. J Infect Dis 2013;**207**(5):842-7.
77. Bhattarai A, Ali AS, Kachur SP, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med 2007;**4**(11):e309.
78. Abeku TA, Kristan M, Jones C, et al. Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. Malaria journal 2008;**7**:202.
79. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings--can they find the last parasite? Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2011;**17**(11):1624-31.
80. Bisoffi Z, Sirima BS, Angheben A, et al. Rapid malaria diagnostic tests vs. clinical management of malaria in rural Burkina Faso: safety and effect on clinical decisions. A randomized trial. Tropical medicine & international health : TM & IH 2009;**14**(5):491-8.
81. Chinkhumba J, Skarbinski J, Chilima B, et al. Comparative field performance and adherence to test results of four malaria rapid diagnostic tests among febrile patients more than five years of age in Blantyre, Malawi. Malaria journal 2010;**9**:209.
82. Nicastri E, Bevilacqua N, Sane Schepisi M, et al. Accuracy of malaria diagnosis by microscopy, rapid diagnostic test, and PCR methods and evidence of antimalarial overprescription in non-severe febrile patients in two Tanzanian hospitals. The American journal of tropical medicine and hygiene 2009;**80**(5):712-7.
83. Martensson A, Stromberg J, Sisowath C, et al. Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood Plasmodium falciparum malaria in Zanzibar, Tanzania. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2005;**41**(8):1079-86.
84. Conroy AL, Hawkes M, Hayford K, et al. Prospective validation of pediatric disease severity scores to predict mortality in Ugandan children presenting with malaria and non-malaria febrile illness. Crit Care 2015;**19**:47.

85. Uzochukwu BS, Obikeze EN, Onwujekwe OE, et al. Cost-effectiveness analysis of rapid diagnostic test, microscopy and syndromic approach in the diagnosis of malaria in Nigeria: implications for scaling-up deployment of ACT. Malar J 2009;**8**:265.
86. Muro F, Reyburn R, Reyburn H. Acute respiratory infection and bacteraemia as causes of non-malarial febrile illness in African children: a narrative review. Pneumonia (Nathan) 2015;**6**:6-17.
87. Biggs HM, Lester R, Nadjm B, et al. Invasive Salmonella infections in areas of high and low malaria transmission intensity in Tanzania. Clin Infect Dis 2014;**58**(5):638-47.
88. Onchiri FM, Pavlinac PB, Singa BO, et al. Low Bacteremia Prevalence Among Febrile Children in Areas of Differing Malaria Transmission in Rural Kenya: A Cross-Sectional Study. J Pediatric Infect Dis Soc 2016;**5**(4):385-94.
89. Onchiri FM, Pavlinac PB, Singa BO, et al. Frequency and correlates of malaria over-treatment in areas of differing malaria transmission: a cross-sectional study in rural Western Kenya. Malar J 2015;**14**:97.
90. Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ 2004;**329**(7476):1212.
91. Gwer S, Newton CR, Berkley JA. Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. Am J Trop Med Hyg 2007;**77**(6 Suppl):6-13.
92. O'Dempsey TJ, McArdle TF, Laurence BE, et al. Overlap in the clinical features of pneumonia and malaria in African children. Trans R Soc Trop Med Hyg 1993;**87**(6):662-5.
93. Kallander K, Nsungwa-Sabiiti J, Peterson S. Symptom overlap for malaria and pneumonia--policy implications for home management strategies. Acta Trop 2004;**90**(2):211-4.
94. Fitzner J, Qasmieh S, Mounts AW, et al. Revision of clinical case definitions: influenza-like illness and severe acute respiratory infection. Bull World Health Organ 2018;**96**(2):122-28.
95. Muller DA, Depelsenaire AC, Young PR. Clinical and Laboratory Diagnosis of Dengue Virus Infection. J Infect Dis 2017;**215**(suppl_2):S89-S95.
96. Dietz VJ, Gubler DJ, Rigau-Perez JG, et al. Epidemic dengue 1 in Brazil, 1986: evaluation of a clinically based dengue surveillance system. Am J Epidemiol 1990;**131**(4):693-701.
97. Nsutebu EF, Ndumbe PM, Koulla S. The increase in occurrence of typhoid fever in Cameroon: overdiagnosis due to misuse of the Widal test? Trans R Soc Trop Med Hyg 2002;**96**(1):64-7.
98. Thriemer K, Ley B, Ame SS, et al. Clinical and epidemiological features of typhoid fever in Pemba, Zanzibar: assessment of the performance of the WHO case definitions. PLoS One 2012;**7**(12):e51823.
99. Luxemburger C, Nosten F, Kyle DE, et al. Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living

in an area of low transmission. Trans R Soc Trop Med Hyg 1998;**92**(1):45-9.
100. Braga JU, Bressan C, Dalvi APR, et al. Accuracy of Zika virus disease case definition during simultaneous Dengue and Chikungunya epidemics. PLoS One 2017;**12**(6):e0179725.
101. Cardoso MR, Nascimento-Carvalho CM, Ferrero F, et al. Adding fever to WHO criteria for diagnosing pneumonia enhances the ability to identify pneumonia cases among wheezing children. Arch Dis Child 2011;**96**(1):58-61.
102. De Santis O, Kilowoko M, Kyungu E, et al. Predictive value of clinical and laboratory features for the main febrile diseases in children living in Tanzania: A prospective observational study. PLoS One 2017;**12**(5):e0173314.
103. Jones AH, Ampofo W, Akuffo R, et al. Sentinel surveillance for influenza among severe acute respiratory infection and acute febrile illness inpatients at three hospitals in Ghana. Influenza Other Respir Viruses 2016;**10**(5):367-74.
104. Gessner BD, Shindo N, Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. Lancet Infect Dis 2011;**11**(3):223-35.
105. Murray EL, Khagayi S, Ope M, et al. What are the most sensitive and specific sign and symptom combinations for influenza in patients hospitalized with acute respiratory illness? Results from western Kenya, January 2007-July 2010. Epidemiol Infect 2013;**141**(1):212-22.
106. Rha B, Dahl RM, Moyes J, et al. Performance of Surveillance Case Definitions in Detecting Respiratory Syncytial Virus Infection Among Young Children Hospitalized With Severe Respiratory Illness-South Africa, 2009-2014. J Pediatric Infect Dis Soc 2018.
107. Farag TH, Nasrin D, Wu Y, et al. Some epidemiologic, clinical, microbiologic, and organizational assumptions that influenced the design and performance of the Global Enteric Multicenter Study (GEMS). Clin Infect Dis 2012;**55 Suppl 4**:S225-31.
108. Kotloff KL, Blackwelder WC, Nasrin D, et al. The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case/control study. Clin Infect Dis 2012;**55 Suppl 4**:S232-45.
109. Kidane G, Morrow RH. Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. Lancet 2000;**356**(9229):550-5.
110. Sazawal S, Black RE. Effect of pneumonia case management on mortality in neonates, infants, and preschool children: a meta-analysis of community-based trials. Lancet Infect Dis 2003;**3**(9):547-56.
111. WHO and United Nations Children Fund. Integrated Management of Childhood Illnesses. Document available at: http://www.who.int/child_adolescent_health/documents/IMCI_chartbooklet/en/index.html, 2008.

112. Weber MW, Mulholland EK, Jaffar S, et al. Evaluation of an algorithm for the integrated management of childhood illness in an area with seasonal malaria in the Gambia. Bull World Health Organ 1997;**75 Suppl 1**:25-32.
113. Gove S. Integrated management of childhood illness by outpatient health workers: technical basis and overview. The WHO Working Group on Guidelines for Integrated Management of the Sick Child. Bull World Health Organ 1997;**75 Suppl 1**:7-24.
114. Tulloch J. Integrated approach to child health in developing countries. Lancet 1999;**354 Suppl 2**:SII16-20.
115. Baynes C, Mboya D, Likasi S, et al. Quality of Sick Child-Care Delivered by Community Health Workers in Tanzania. Int J Health Policy Manag 2018;**7**(12):1097-109.
116. Gove S, Tamburlini G, Molyneux E, et al. Development and technical basis of simplified guidelines for emergency triage assessment and treatment in developing countries. WHO Integrated Management of Childhood Illness (IMCI) Referral Care Project. Arch Dis Child 1999;**81**(6):473-7.
117. Robertson SK, Manson K, Fioratou E. IMCI and ETAT integration at a primary healthcare facility in Malawi: a human factors approach. BMC Health Serv Res 2018;**18**(1):1014.
118. Yeboah-Antwi K, Pilingana P, Macleod WB, et al. Community case management of fever due to malaria and pneumonia in children under five in Zambia: a cluster randomized controlled trial. PLoS Med 2010;**7**(9):e1000340.
119. Mukanga D, Tiono AB, Anyorigiya T, et al. Integrated community case management of fever in children under five using rapid diagnostic tests and respiratory rate counting: a multi-country cluster randomized trial. Am J Trop Med Hyg 2012;**87**(5 Suppl):21-29.
120. Pandya H, Slemming W, Saloojee H. Health system factors affecting implementation of integrated management of childhood illness (IMCI): qualitative insights from a South African province. Health Policy Plan 2018;**33**(2):171-82.
121. Baiden F, Owusu-Agyei S, Bawah J, et al. An evaluation of the clinical assessments of under-five febrile children presenting to primary health facilities in rural Ghana. PLoS One 2011;**6**(12):e28944.
122. Uwemedimo OT, Lewis TP, Essien EA, et al. Distribution and determinants of pneumonia diagnosis using Integrated Management of Childhood Illness guidelines: a nationally representative study in Malawi. BMJ Glob Health 2018;**3**(2):e000506.
123. Kundra S, Singh T, Chhatwal J. Utility of Indian adaptation of Integrated Management of Childhood Illness (IMCI) algorithm. Indian J Pediatr 2008;**75**(8):781-5.
124. Gupta R, Sachdev HP, Shah D. Evaluation of the WHO/UNICEF algorithm for integrated management of childhood illness between the ages of one week to two months. Indian Pediatr 2000;**37**(4):383-90.

125. Shah D, Sachdev HP. Evaluation of the WHO/UNICEF algorithm for integrated management of childhood illness between the age of two months to five years. Indian Pediatr 1999;**36**(8):767-77.
126. Chowdhury EK, El Arifeen S, Rahman M, et al. Care at first-level facilities for children with severe pneumonia in Bangladesh: a cohort study. Lancet 2008;**372**(9641):822-30.
127. Nnedu ON, Rimel B, Terry C, et al. Syndromic diagnosis of malaria in rural Sierra Leone and proposed additions to the national integrated management of childhood illness guidelines for fever. Am J Trop Med Hyg 2010;**82**(4):525-8.
128. Nguyen DT, Leung KK, McIntyre L, et al. Does integrated management of childhood illness (IMCI) training improve the skills of health workers? A systematic review and meta-analysis. PLoS One 2013;**8**(6):e66030.
129. Gera T, Shah D, Garner P, et al. Integrated management of childhood illness (IMCI) strategy for children under five. Cochrane Database Syst Rev 2016(6):CD010123.
130. Sahin F, Ulukol B, Aysev D, et al. The validity of diagnostic criteria for streptococcal pharyngitis in Integrated Management of Childhood Illness (IMCI) guidelines. J Trop Pediatr 2003;**49**(6):377-9.
131. Armstrong Schellenberg JR, Adam T, Mshinda H, et al. Effectiveness and cost of facility-based Integrated Management of Childhood Illness (IMCI) in Tanzania. Lancet 2004;**364**(9445):1583-94.
132. Arifeen SE, Hoque DM, Akter T, et al. Effect of the Integrated Management of Childhood Illness strategy on childhood mortality and nutrition in a rural area in Bangladesh: a cluster randomised trial. Lancet 2009;**374**(9687):393-403.
133. Okeke IN, Klugman KP, Bhutta ZA, et al. Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect Dis 2005;**5**(9):568-80.
134. Gouws E, Bryce J, Habicht JP, et al. Improving antimicrobial use among health workers in first-level facilities: results from the multi-country evaluation of the Integrated Management of Childhood Illness strategy. Bull World Health Organ 2004;**82**(7):509-15.
135. Abeid KA, Jani B, Cortese MM, et al. Monovalent Rotavirus Vaccine Effectiveness and Impact on Rotavirus Hospitalizations in Zanzibar, Tanzania: Data From the First 3 Years After Introduction. J Infect Dis 2017;**215**(2):183-91.
136. Ngocho JS, Magoma B, Olomi GA, et al. Effectiveness of pneumococcal conjugate vaccines against invasive pneumococcal disease among children under five years of age in Africa: A systematic review. PLoS One 2019;**14**(2):e0212295.
137. Bryce J, Victora CG, Habicht JP, et al. Programmatic pathways to child survival: results of a multi-country evaluation of Integrated Management of Childhood Illness. Health Policy Plan 2005;**20 Suppl 1**:i5-i17.
138. Zanzibar Ministry of Health Z. Annual Health Information Bulletin, 2010.

139. Campbell H, Byass P, Lamont AC, et al. Assessment of clinical criteria for identification of severe acute lower respiratory tract infections in children. Lancet 1989;**1**(8633):297-9.
140. Harris M, Clark J, Coote N, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax 2011;**66 Suppl 2**:ii1-23.
141. Esposito S. Management of community-acquired pneumonia in infants and children older than 3 months. Clin Infect Dis 2012;**54**(6):884-5; author reply 85.
142. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis 2011;**53**(7):e25-76.
143. Lynch T, Bialy L, Kellner JD, et al. A systematic review on the diagnosis of pediatric bacterial pneumonia: when gold is bronze. PLoS One 2010;**5**(8):e11989.
144. Hazir T, Nisar YB, Qazi SA, et al. Chest radiography in children aged 2-59 months diagnosed with non-severe pneumonia as defined by World Health Organization: descriptive multicentre study in Pakistan. BMJ 2006;**333**(7569):629.
145. Nascimento-Carvalho CM, Madhi SA, O'Brien KL. Review of guidelines for evidence-based management for childhood community-acquired pneumonia in under-5 years from developed and developing countries. Pediatr Infect Dis J 2013;**32**(11):1281-2.
146. Mulholland EK, Simoes EA, Costales MO, et al. Standardized diagnosis of pneumonia in developing countries. Pediatr Infect Dis J 1992;**11**(2):77-81.
147. Wingerter SL, Bachur RG, Monuteaux MC, et al. Application of the world health organization criteria to predict radiographic pneumonia in a US-based pediatric emergency department. Pediatr Infect Dis J 2012;**31**(6):561-4.
148. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bull World Health Organ 2005;**83**(5):353-9.
149. Dapas JQ, D. Antimicrobial Stewardship in Low- and Middle-Income Countries. Current Treatment Options in Infectious Diseases 2018.
150. Eurosurveillance editorial t. WHO member states adopt global action plan on antimicrobial resistance. Euro Surveill 2015;**20**(21).
151. Frimpong G, Ofori-Kwakye K, Kuntworbe N, et al. Quality Assessment of Some Essential Children's Medicines Sold in Licensed Outlets in Ashanti Region, Ghana. J Trop Med 2018;**2018**:1494957.
152. Cox JA, Vlieghe E, Mendelson M, et al. Antibiotic stewardship in low- and middle-income countries: the same but different? Clin Microbiol Infect 2017;**23**(11):812-18.
153. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance-the need for global solutions. Lancet Infect Dis 2013;**13**(12):1057-98.

154. Nadimpalli M, Delarocque-Astagneau E, Love DC, et al. Combating Global Antibiotic Resistance: Emerging One Health Concerns in Lower- and Middle-Income Countries. Clin Infect Dis 2018;**66**(6):963-69.
155. Mosha JF, Conteh L, Tediosi F, et al. Cost implications of improving malaria diagnosis: findings from north-eastern Tanzania. PLoS One 2010;**5**(1):e8707.
156. Nichols C, Cruz Espinoza LM, von Kalckreuth V, et al. Bloodstream Infections and Frequency of Pretreatment Associated With Age and Hospitalization Status in Sub-Saharan Africa. Clin Infect Dis 2015;**61 Suppl 4**:S372-9.
157. Marks F, von Kalckreuth V, Aaby P, et al. Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study. Lancet Glob Health 2017;**5**(3):e310-e23.
158. Mahende C, Ngasala B, Lusingu J, et al. Aetiology of acute febrile episodes in children attending korogwe district hospital in north-eastern Tanzania. PLoS One 2014;**9**(8):e104197.
159. Thriemer K, Ley B, Ame S, et al. The burden of invasive bacterial infections in Pemba, Zanzibar. PLoS One 2012;**7**(2):e30350.
160. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis 2010;**10**(6):417-32.
161. D'Acremont V, Kilowoko M, Kyungu E, et al. Beyond malaria--causes of fever in outpatient Tanzanian children. N Engl J Med 2014;**370**(9):809-17.
162. Kibuuka A, Byakika-Kibwika P, Achan J, et al. Bacteremia Among Febrile Ugandan Children Treated with Antimalarials Despite a Negative Malaria Test. Am J Trop Med Hyg 2015;**93**(2):276-80.
163. Schwarz NG, Sarpong N, Hunger F, et al. Systemic bacteraemia in children presenting with clinical pneumonia and the impact of non-typhoid salmonella (NTS). BMC Infect Dis 2010;**10**:319.
164. Hildenwall H, Amos B, Mtove G, et al. Causes of non-malarial febrile illness in outpatients in Tanzania. Trop Med Int Health 2016;**21**(1):149-56.
165. Brent AJ, Ahmed I, Ndiritu M, et al. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. Lancet 2006;**367**(9509):482-8.
166. Lo SW, Gladstone RA, van Tonder AJ, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. Lancet Infect Dis 2019.
167. Mackenzie GA, Hill PC, Jeffries DJ, et al. Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a population-based surveillance study. Lancet Infect Dis 2016;**16**(6):703-11.
168. Mtove G, Amos B, von Seidlein L, et al. Invasive salmonellosis among children admitted to a rural Tanzanian hospital and a comparison with previous studies. PLoS One 2010;**5**(2):e9244.

169. Rackoff LA, Bok K, Green KY, et al. Epidemiology and evolution of rotaviruses and noroviruses from an archival WHO Global Study in Children (1976-79) with implications for vaccine design. PLoS One 2013;**8**(3):e59394.
170. Wang S-M, Ma J-C, Hao Z-Y, et al. Surveillance of shigellosis by real-time PCR suggests underestimation of shigellosis prevalence by culture-based methods in a population of rural China. Journal of Infection 2010;**61**(6):471-75.
171. Sinha A, Sengupta S, Guin S, et al. Culture-independent real-time PCR reveals extensive polymicrobial infections in hospitalized diarrhoea cases in Kolkata, India. Clin Microbiol Infect 2011.
172. Chang HY, Chang LY, Shao PL, et al. Comparison of real-time polymerase chain reaction and serological tests for the confirmation of Mycoplasma pneumoniae infection in children with clinical diagnosis of atypical pneumonia. J Microbiol Immunol Infect 2014;**47**(2):137-44.
173. Spuesens EB, Fraaij PL, Visser EG, et al. Carriage of Mycoplasma pneumoniae in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med 2013;**10**(5):e1001444.
174. de Boer RF, Ott A, Kesztyus B, et al. Improved detection of five major gastrointestinal pathogens by use of a molecular screening approach. J Clin Microbiol 2010;**48**(11):4140-6.
175. Wolffs PFG, Bruggeman CA, van Well GTJ, et al. Replacing traditional diagnostics of fecal viral pathogens by a comprehensive panel of real-time PCRs. Journal of clinical microbiology 2011;**49**(5):1926-31.
176. McNally LM, Jeena PM, Gajee K, et al. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. Lancet 2007;**369**(9571):1440-51.
177. Bodhidatta L, McDaniel P, Sornsakrin S, et al. Case-control study of diarrheal disease etiology in a remote rural area in Western Thailand. Am J Trop Med Hyg 2010;**83**(5):1106-9.
178. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet 2016;**388**(10051):1291-301.
179. Fry AM, Lu X, Olsen SJ, et al. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. PLoS One 2011;**6**(3):e17780.
180. Feikin DR, Njenga MK, Bigogo G, et al. Etiology and Incidence of viral and bacterial acute respiratory illness among older children and adults in rural western Kenya, 2007-2010. PLoS One 2012;**7**(8):e43656.
181. Jansen RR, Wieringa J, Koekkoek SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. J Clin Microbiol 2011;**49**(7):2631-6.
182. El Kholy AA, Mostafa NA, Ali AA, et al. The use of multiplex PCR for the diagnosis of viral severe acute respiratory infection in children: a high rate

of co-detection during the winter season. Eur J Clin Microbiol Infect Dis 2016;**35**(10):1607-13.
183. Debiaggi M, Canducci F, Ceresola ER, et al. The role of infections and coinfections with newly identified and emerging respiratory viruses in children. Virol J 2012;**9**:247.
184. Thorburn F, Bennett S, Modha S, et al. The use of next generation sequencing in the diagnosis and typing of respiratory infections. J Clin Virol 2015;**69**:96-100.
185. Hercik C, Cosmas L, Mogeni OD, et al. A diagnostic and epidemiologic investigation of acute febrile illness (AFI) in Kilombero, Tanzania. PLoS One 2017;**12**(12):e0189712.
186. Platts-Mills JA, Liu J, Rogawski ET, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. Lancet Glob Health 2018;**6**(12):e1309-e18.
187. Phillips G, Lopman B, Tam CC, et al. Diagnosing rotavirus A associated IID: Using ELISA to identify a cut-off for real time RT-PCR. J Clin Virol 2009;**44**(3):242-5.
188. Phillips G, Lopman B, Tam CC, et al. Diagnosing norovirus-associated infectious intestinal disease using viral load. BMC Infect Dis 2009;**9**:63.
189. Dung TT, Phat VV, Nga TV, et al. The validation and utility of a quantitative one-step multiplex RT real-time PCR targeting rotavirus A and norovirus. J Virol Methods 2013;**187**(1):138-43.
190. Lindsay B, Ochieng JB, Ikumapayi UN, et al. Quantitative PCR for Detection of Shigella Improves Ascertainment of Shigella Burden in Children with Moderate-to-Severe Diarrhea in Low-Income Countries. J Clin Microbiol 2013;**51**(6):1740-6.
191. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis 2010;**201**(11):1625-32.
192. Houben ML, Coenjaerts FE, Rossen JW, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. J Med Virol 2010;**82**(7):1266-71.
193. Scagnolari C, Midulla F, Selvaggi C, et al. Evaluation of viral load in infants hospitalized with bronchiolitis caused by respiratory syncytial virus. Med Microbiol Immunol 2012;**201**(3):311-7.
194. Kais M, Spindler C, Kalin M, et al. Quantitative detection of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis in lower respiratory tract samples by real-time PCR. Diagn Microbiol Infect Dis 2006;**55**(3):169-78.
195. Abdeldaim GM, Stralin K, Kirsebom LA, et al. Detection of Haemophilus influenzae in respiratory secretions from pneumonia patients by quantitative real-time polymerase chain reaction. Diagn Microbiol Infect Dis 2009;**64**(4):366-73.

196. Greiner O, Day PJ, Bosshard PP, et al. Quantitative detection of Streptococcus pneumoniae in nasopharyngeal secretions by real-time PCR. J Clin Microbiol 2001;**39**(9):3129-34.
197. Fuller JA, Njenga MK, Bigogo G, et al. Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity, Kenya. J Med Virol 2013;**85**(5):924-32.
198. Wishaupt JO, Ploeg TV, Smeets LC, et al. Pitfalls in interpretation of CT-values of RT-PCR in children with acute respiratory tract infections. J Clin Virol 2017;**90**:1-6.
199. Bolotin S, Deeks SL, Marchand-Austin A, et al. Correlation of Real Time PCR Cycle Threshold Cut-Off with Bordetella pertussis Clinical Severity. PLoS One 2015;**10**(7):e0133209.
200. DeVincenzo JP, Guyton C, Rea H, et al. Molecular detection and quantification of pertussis and correlation with clinical outcomes in children. Diagn Microbiol Infect Dis 2013;**76**(1):10-5.
201. Kovacs SD, Mullholland K, Bosch J, et al. Deconstructing the differences: a comparison of GBD 2010 and CHERG's approach to estimating the mortality burden of diarrhea, pneumonia, and their etiologies. BMC Infect Dis 2015;**15**:16.
202. Blackwelder WC, Biswas K, Wu Y, et al. Statistical methods in the Global Enteric Multicenter Study (GEMS). Clin Infect Dis 2012;**55 Suppl 4**:S246-53.
203. Hammitt LL, Feikin DR, Scott JAG, et al. Addressing the Analytic Challenges of Cross-Sectional Pediatric Pneumonia Etiology Data. Clin Infect Dis 2017;**64**(suppl_3):S197-S204.
204. Scheltema NM, Gentile A, Lucion F, et al. Global respiratory syncytial virus-associated mortality in young children (RSV GOLD): a retrospective case series. Lancet Glob Health 2017;**5**(10):e984-e91.
205. Veiga MI, Ferreira PE, Bjorkman A, et al. Multiplex PCR-RFLP methods for pfcrt, pfmdr1 and pfdhfr mutations in Plasmodium falciparum. Mol Cell Probes 2006;**20**(2):100-4.
206. Holmgren G, Hamrin J, Svard J, et al. Selection of pfmdr1 mutations after amodiaquine monotherapy and amodiaquine plus artemisinin combination therapy in East Africa. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 2007;**7**(5):562-9.
207. Shokoples SE, Ndao M, Kowalewska-Grochowska K, et al. Multiplexed real-time PCR assay for discrimination of Plasmodium species with improved sensitivity for mixed infections. Journal of clinical microbiology 2009;**47**(4):975-80.
208. Hsiang MS, Lin M, Dokomajilar C, et al. PCR-based pooling of dried blood spots for detection of malaria parasites: optimization and application to a cohort of Ugandan children. J Clin Microbiol 2010;**48**(10):3539-43.
209. Baker J, McCarthy J, Gatton M, et al. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the

performance of PfHRP2-based rapid diagnostic tests. The Journal of infectious diseases 2005;**192**(5):870-7.
210. Cheesbrough M. *District Laboratory Practice in Tropical countries*. Cambridge: Co-published by the press syndicate of The University of Cambridge andTropical Health Technology, 2001.
211. Vandepitte J. *Basic laboratory procedures in clinical bacteriology*. 2nd ed. Geneva: World Health Organization, 2003.
212. Mediannikov O, Socolovschi C, Edouard S, et al. Common epidemiology of Rickettsia felis infection and malaria, Africa. Emerg Infect Dis 2013;**19**(11):1775-83.
213. Walter G, Botelho-Nevers E, Socolovschi C, et al. Murine typhus in returned travelers: a report of thirty-two cases. Am J Trop Med Hyg 2012;**86**(6):1049-53.
214. Harris E, Roberts TG, Smith L, et al. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. J Clin Microbiol 1998;**36**(9):2634-9.
215. Huang C, Slater B, Rudd R, et al. First Isolation of West Nile virus from a patient with encephalitis in the United States. Emerg Infect Dis 2002;**8**(12):1367-71.
216. Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. Scand J Infect Dis 2014:1-5.
217. Sansone M, Andersson M, Brittain-Long R, et al. Rhinovirus infections in western Sweden: a four-year molecular epidemiology study comparing local and globally appearing types. Eur J Clin Microbiol Infect Dis 2013;**32**(7):947-54.
218. Van den Bruel A, Thompson MJ, Haj-Hassan T, et al. Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. BMJ 2011;**342**:d3082.
219. . Pocket Book of Hospital Care for Children: Guidelines for the Management of Common Childhood Illnesses. 2nd ed. Geneva, 2013.
220. Nankabirwa J, Brooker SJ, Clarke SE, et al. Malaria in school-age children in Africa: an increasingly important challenge. Trop Med Int Health 2014;**19**(11):1294-309.
221. Cook J, Xu W, Msellem M, et al. Mass screening and treatment on the basis of results of a Plasmodium falciparum-specific rapid diagnostic test did not reduce malaria incidence in Zanzibar. J Infect Dis 2015;**211**(9):1476-83.
222. Morris U, Msellem MI, Mkali H, et al. A cluster randomised controlled trial of two rounds of mass drug administration in Zanzibar, a malaria pre-elimination setting-high coverage and safety, but no significant impact on transmission. BMC Med 2018;**16**(1):215.
223. WHO. Malaria rapid diagnostic test performance: results of WHO product testing malaria RDTs: round 2 (2009). 2010.

224. Gendrot M, Fawaz R, Dormoi J, et al. Genetic diversity and deletion of Plasmodium falciparum histidine-rich protein 2 and 3: a threat to diagnosis of P. falciparum malaria. Clin Microbiol Infect 2019;**25**(5):580-85.
225. Berhane A, Anderson K, Mihreteab S, et al. Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea. Emerg Infect Dis 2018;**24**(3):462-70.
226. Gillet P, Scheirlinck A, Stokx J, et al. Prozone in malaria rapid diagnostics tests: how many cases are missed? Malaria journal 2011;**10**:166.
227. Gillet P, Mori M, Van Esbroeck M, et al. Assessment of the prozone effect in malaria rapid diagnostic tests. Malar J 2009;**8**:271.
228. McMorrow ML, Masanja MI, Abdulla SM, et al. Challenges in routine implementation and quality control of rapid diagnostic tests for malaria--Rufiji District, Tanzania. The American journal of tropical medicine and hygiene 2008;**79**(3):385-90.
229. Burchett HE, Leurent B, Baiden F, et al. Improving prescribing practices with rapid diagnostic tests (RDTs): synthesis of 10 studies to explore reasons for variation in malaria RDT uptake and adherence. BMJ Open 2017;**7**(3):e012973.
230. Batwala V, Magnussen P, Nuwaha F. Are rapid diagnostic tests more accurate in diagnosis of plasmodium falciparum malaria compared to microscopy at rural health centres? Malar J 2010;**9**:349.
231. Ghai RR, Thurber MI, El Bakry A, et al. Multi-method assessment of patients with febrile illness reveals over-diagnosis of malaria in rural Uganda. Malar J 2016;**15**:460.
232. Abdalla ZA, Rahma NA, Hassan EE, et al. The diagnostic performance of rapid diagnostic tests and microscopy for malaria diagnosis in eastern Sudan using a nested polymerase chain reaction assay as a reference standard. Trans R Soc Trop Med Hyg 2019.
233. Mahende C, Ngasala B, Lusingu J, et al. Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe District, Tanzania. Malar J 2016;**15**(1):391.
234. Falade CO, Ajayi IO, Nsungwa-Sabiiti J, et al. Malaria Rapid Diagnostic Tests and Malaria Microscopy for Guiding Malaria Treatment of Uncomplicated Fevers in Nigeria and Prereferral Cases in 3 African Countries. Clin Infect Dis 2016;**63**(suppl 5):S290-S97.
235. Mayxay M, Pukrittayakamee S, Chotivanich K, et al. Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria. Trans R Soc Trop Med Hyg 2001;**95**(2):179-82.
236. Ramesh A, Nakielny S, Hsu J, et al. Metagenomic next-generation sequencing of samples from pediatric febrile illness in Tororo, Uganda. PLoS One 2019;**14**(6):e0218318.
237. Yman V, Wandell G, Mutemi DD, et al. Persistent transmission of Plasmodium malariae and Plasmodium ovale species in an area of declining Plasmodium

falciparum transmission in eastern Tanzania. PLoS Negl Trop Dis 2019;**13**(5):e0007414.
238. Yerlikaya S, Campillo A, Gonzalez IJ. A Systematic Review: Performance of Rapid Diagnostic Tests for the Detection of Plasmodium knowlesi, Plasmodium malariae, and Plasmodium ovale Monoinfections in Human Blood. J Infect Dis 2018;**218**(2):265-76.
239. Orish VN, Ansong JY, Onyeabor OS, et al. Overdiagnosis and overtreatment of malaria in children in a secondary healthcare centre in Sekondi-Takoradi, Ghana. Trop Doct 2016;**46**(4):191-98.
240. Bruxvoort KJ, Leurent B, Chandler CIR, et al. The Impact of Introducing Malaria Rapid Diagnostic Tests on Fever Case Management: A Synthesis of Ten Studies from the ACT Consortium. Am J Trop Med Hyg 2017.
241. Ansah EK, Narh-Bana S, Affran-Bonful H, et al. The impact of providing rapid diagnostic malaria tests on fever management in the private retail sector in Ghana: a cluster randomized trial. BMJ 2015;**350**:h1019.
242. Kiemde F, Bonko MDA, Tahita MC, et al. 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. Malar J 2017;**16**(1):294.
243. Thwing J, Ba F, Diaby A, et al. Assessment of the utility of a symptom-based algorithm for identifying febrile patients for malaria diagnostic testing in Senegal. Malar J 2017;**16**(1):95.
244. Staedke SG, Maiteki-Sebuguzi C, DiLiberto DD, et al. The Impact of an Intervention to Improve Malaria Care in Public Health Centers on Health Indicators of Children in Tororo, Uganda (PRIME): A Cluster-Randomized Trial. Am J Trop Med Hyg 2016;**95**(2):358-67.
245. Chandler CI, Webb EL, Maiteki-Sebuguzi C, et al. The impact of an intervention to introduce malaria rapid diagnostic tests on fever case management in a high transmission setting in Uganda: A mixed-methods cluster-randomized trial (PRIME). PLoS One 2017;**12**(3):e0170998.
246. Investigators M-EN. The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. Clin Infect Dis 2014;**59 Suppl 4**:S193-206.
247. Feikin DR, Njenga MK, Bigogo G, et al. Viral and bacterial causes of severe acute respiratory illness among children aged less than 5 years in a high malaria prevalence area of western Kenya, 2007-2010. Pediatr Infect Dis J 2013;**32**(1):e14-9.
248. Berkley JA, Munywoki P, Ngama M, et al. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA 2010;**303**(20):2051-7.
249. O'Meara WP, Mott JA, Laktabai J, et al. Etiology of pediatric fever in western Kenya: a case-control study of falciparum malaria, respiratory viruses, and streptococcal pharyngitis. Am J Trop Med Hyg 2015;**92**(5):1030-7.

250. Hammitt LL, Kazungu S, Morpeth SC, et al. A preliminary study of pneumonia etiology among hospitalized children in Kenya. Clin Infect Dis 2012;**54 Suppl 2**:S190-9.
251. Wu Z, Deloria-Knoll M, Zeger SL. Nested partially latent class models for dependent binary data; estimating disease etiology. Biostatistics 2017;**18**(2):200-13.
252. Shi T, McLean K, Campbell H, et al. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta-analysis. J Glob Health 2015;**5**(1):010408.
253. Feikin DR, Fu W, Park DE, et al. Is Higher Viral Load in the Upper Respiratory Tract Associated With Severe Pneumonia? Findings From the PERCH Study. Clin Infect Dis 2017;**64**(suppl_3):S337-S46.
254. Benet T, Picot VS, Awasthi S, et al. Severity of Pneumonia in Under 5-Year-Old Children from Developing Countries: A Multicenter, Prospective, Observational Study. Am J Trop Med Hyg 2017;**97**(1):68-76.
255. Rhedin S, Lindstrand A, Hjelmgren A, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax 2015;**70**(9):847-53.
256. Colvin JM, Muenzer JT, Jaffe DM, et al. Detection of viruses in young children with fever without an apparent source. Pediatrics 2012;**130**(6):e1455-62.
257. Lau SK, Yip CC, Tsoi HW, et al. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 2007;**45**(11):3655-64.
258. Morobe JM, Nyiro JU, Brand S, et al. Human rhinovirus spatial-temporal epidemiology in rural coastal Kenya, 2015-2016, observed through outpatient surveillance. Wellcome Open Res 2018;**3**:128.
259. Higdon MM, Hammitt LL, Deloria Knoll M, et al. Should Controls With Respiratory Symptoms Be Excluded From Case-Control Studies of Pneumonia Etiology? Reflections From the PERCH Study. Clin Infect Dis 2017;**64**(suppl_3):S205-S12.
260. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;**381**(9875):1405-16.
261. Forsell J, Granlund M, Samuelsson L, et al. High occurrence of Blastocystis sp. subtypes 1-3 and Giardia intestinalis assemblage B among patients in Zanzibar, Tanzania. Parasit Vectors 2016;**9**(1):370.
262. Haque R, Roy S, Siddique A, et al. Multiplex real-time PCR assay for detection of Entamoeba histolytica, Giardia intestinalis, and Cryptosporidium spp. Am J Trop Med Hyg 2007;**76**(4):713-7.
263. Checkley W, White AC, Jr., Jaganath D, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis 2015;**15**(1):85-94.

264. Crump JA, Morrissey AB, Nicholson WL, et al. Etiology of Severe Non-malaria Febrile Illness in Northern Tanzania: A Prospective Cohort Study. PLoS Negl Trop Dis 2013;**7**(7):e2324.
265. Katz MA, Lebo E, Emukule G, et al. Epidemiology, seasonality, and burden of influenza and influenza-like illness in urban and rural Kenya, 2007-2010. J Infect Dis 2012;**206 Suppl 1**:S53-60.
266. Sokhna C, Mediannikov O, Fenollar F, et al. Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl Trop Dis 2013;**7**(1):e1999.
267. Caserta MT, McDermott MP, Dewhurst S, et al. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. J Pediatr 2004;**145**(4):478-84.
268. Caserta MT, Hall CB, Schnabel K, et al. Diagnostic assays for active infection with human herpesvirus 6 (HHV-6). J Clin Virol 2010;**48**(1):55-7.
269. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. Clin Microbiol Rev 2015;**28**(2):313-35.
270. Howard LM, Mwape I, Siwingwa M, et al. Norovirus infections in young children in Lusaka Province, Zambia: clinical characteristics and molecular epidemiology. BMC Infect Dis 2017;**17**(1):92.
271. Narkeviciute I, Tamusauskaite I. Peculiarities of norovirus and rotavirus infections in hospitalised young children. J Pediatr Gastroenterol Nutr 2008;**46**(3):289-92.
272. Leuthard D, Berger C, Staubli G, et al. Management of Children with Travel-related Illness Evaluated in a Pediatric Emergency Room. Pediatr Infect Dis J 2015;**34**(12):1279-82.
273. Naudin J, Blonde R, Alberti C, et al. Aetiology and epidemiology of fever in children presenting to the emergency department of a French paediatric tertiary care centre after international travel. Arch Dis Child 2012;**97**(2):107-11.
274. Hagmann S, Neugebauer R, Schwartz E, et al. Illness in children after international travel: analysis from the GeoSentinel Surveillance Network. Pediatrics 2010;**125**(5):e1072-80.
275. Leder K, Torresi J, Libman MD, et al. GeoSentinel surveillance of illness in returned travelers, 2007-2011. Ann Intern Med 2013;**158**(6):456-68.
276. Wilson ME, Weld LH, Boggild A, et al. Fever in returned travelers: results from the GeoSentinel Surveillance Network. Clin Infect Dis 2007;**44**(12):1560-8.
277. Parola P, Paddock CD, Socolovschi C, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev 2013;**26**(4):657-702.
278. Vairo F, Nicastri E, Meschi S, et al. Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. Int J Infect Dis 2012;**16**(1):e44-6.
279. Hertz JT, Munishi OM, Ooi EE, et al. Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. Am J Trop Med Hyg 2012;**86**(1):171-7.

280. Ward T, Samuel M, Maoz D, et al. Dengue data and surveillance in Tanzania: a systematic literature review. Trop Med Int Health 2017;**22**(8):960-70.
281. Tarnagda Z, Cisse A, Bicaba BW, et al. Dengue Fever in Burkina Faso, 2016. Emerg Infect Dis 2018;**24**(1):170-72.
282. Hashimoto T, Kutsuna S, Maeki T, et al. A Case of Dengue Fever Imported from Burkina Faso to Japan in October 2016. Jpn J Infect Dis 2017;**70**(6):675-77.
283. Amarasinghe A, Kuritsk JN, Letson GW, et al. Dengue virus infection in Africa. Emerg Infect Dis 2011;**17**(8):1349-54.
284. Ferede G, Tiruneh M, Abate E, et al. A study of clinical, hematological, and biochemical profiles of patients with dengue viral infections in Northwest Ethiopia: implications for patient management. BMC Infect Dis 2018;**18**(1):616.
285. Teoh BT, Sam SS, Tan KK, et al. The Use of NS1 Rapid Diagnostic Test and qRT-PCR to Complement IgM ELISA for Improved Dengue Diagnosis from Single Specimen. Sci Rep 2016;**6**:27663.
286. Toro C, Trevisi P, Lopez-Quintana B, et al. Imported Dengue Infection in a Spanish Hospital with a High Proportion of Travelers from Africa: A 9-Year Retrospective Study. Am J Trop Med Hyg 2017;**96**(3):701-07.
287. Overbosch FW, Schinkel J, Stolte IG, et al. Dengue virus infection among long-term travelers from the Netherlands: A prospective study, 2008-2011. PLoS One 2018;**13**(2):e0192193.
288. Lim JK, Carabali M, Lee JS, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open 2018;**8**(1):e017673.
289. Chipwaza B, Mugasa JP, Selemani M, et al. Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. PLoS Negl Trop Dis 2014;**8**(11):e3335.
290. Vu DM, Mutai N, Heath CJ, et al. Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014-2015. Emerg Infect Dis 2017;**23**(11):1915-17.
291. Kolawole OM, Seriki AA, Irekeola AA, et al. Dengue virus and malaria concurrent infection among febrile subjects within Ilorin metropolis, Nigeria. J Med Virol 2017;**89**(8):1347-53.
292. Oludele J, Lesko B, Mahumane Gundane I, et al. Dengue Virus Serotype 2 Established in Northern Mozambique (2015-2016). Am J Trop Med Hyg 2017;**97**(5):1418-22.
293. Capeding MR, Chua MN, Hadinegoro SR, et al. Dengue and other common causes of acute febrile illness in Asia: an active surveillance study in children. PLoS Negl Trop Dis 2013;**7**(7):e2331.
294. Wu X, Lu Y, Zhou S, et al. Impact of climate change on human infectious diseases: Empirical evidence and human adaptation. Environ Int 2016;**86**:14-23.

295. Mourembou G, Lekana-Douki JB, Mediannikov O, et al. Possible Role of Rickettsia felis in Acute Febrile Illness among Children in Gabon. Emerg Infect Dis 2015;**21**(10):1808-15.
296. Toure M, Petersen PT, Bathily SN, et al. Molecular Evidence of Malaria and Zoonotic Diseases Among Rapid Diagnostic Test-Negative Febrile Patients in Low-Transmission Season, Mali. Am J Trop Med Hyg 2017;**96**(2):335-37.
297. Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. J Biosci 2008;**33**(4):557-69.
298. Maina AN, Farris CM, Odhiambo A, et al. Q Fever, Scrub Typhus, and Rickettsial Diseases in Children, Kenya, 2011-2012. Emerg Infect Dis 2016;**22**(5):883-6.
299. Bouley AJ, Biggs HM, Stoddard RA, et al. Brucellosis among hospitalized febrile patients in northern Tanzania. Am J Trop Med Hyg 2012;**87**(6):1105-11.
300. Munywoki PK, Koech DC, Agoti CN, et al. Influence of age, severity of infection, and co-infection on the duration of respiratory syncytial virus (RSV) shedding. Epidemiol Infect 2014:1-9.
301. Okiro EA, White LJ, Ngama M, et al. Duration of shedding of respiratory syncytial virus in a community study of Kenyan children. BMC Infect Dis 2010;**10**:15.
302. Lau LL, Cowling BJ, Fang VJ, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis 2010;**201**(10):1509-16.
303. Loeb M, Singh PK, Fox J, et al. Longitudinal study of influenza molecular viral shedding in Hutterite communities. J Infect Dis 2012;**206**(7):1078-84.
304. Takeyama A, Hashimoto K, Sato M, et al. Respiratory syncytial virus shedding by children hospitalized with lower respiratory tract infection. J Med Virol 2016;**88**(6):938-46.
305. Bhattarai A, Villanueva J, Palekar RS, et al. Viral shedding duration of pandemic influenza A H1N1 virus during an elementary school outbreak--Pennsylvania, May-June 2009. Clin Infect Dis 2011;**52 Suppl 1**:S102-8.
306. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season. J Med Virol 2006;**78**(5):644-50.
307. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A Virus Shedding and Infectivity in Households. J Infect Dis 2015;**212**(9):1420-8.
308. Chen X, Pouran Yousef K, Duwe S, et al. Quantitative influenza follow-up testing (QIFT)--a novel biomarker for the monitoring of disease activity at the point-of-care. PLoS One 2014;**9**(3):e92500.
309. Regamey N, Kaiser L, Roiha HL, et al. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. Pediatr Infect Dis J 2008;**27**(2):100-5.

310. Loeffelholz MJ, Trujillo R, Pyles RB, et al. Duration of rhinovirus shedding in the upper respiratory tract in the first year of life. Pediatrics 2014;**134**(6):1144-50.
311. Chen WJ, Arnold JC, Fairchok MP, et al. Epidemiologic, clinical, and virologic characteristics of human rhinovirus infection among otherwise healthy children and adults: rhinovirus among adults and children. J Clin Virol 2015;**64**:74-82.
312. Martinez ME. The calendar of epidemics: Seasonal cycles of infectious diseases. PLoS Pathog 2018;**14**(11):e1007327.
313. Carneiro I, Roca-Feltrer A, Griffin JT, et al. Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: a systematic review and pooled analysis. PLoS One 2010;**5**(2):e8988.
314. Obando-Pacheco P, Justicia-Grande AJ, Rivero-Calle I, et al. Respiratory Syncytial Virus Seasonality: A Global Overview. J Infect Dis 2018;**217**(9):1356-64.
315. Althouse BM, Flasche S, Minh LN, et al. Seasonality of respiratory viruses causing hospitalizations for acute respiratory infections in children in Nha Trang, Vietnam. Int J Infect Dis 2018;**75**:18-25.
316. Levine H, Mimouni D, Zurel-Farber A, et al. Time trends of viral meningitis among young adults in Israel: 1978-2012. Eur J Clin Microbiol Infect Dis 2014;**33**(7):1149-53.
317. Patel MM, Pitzer VE, Alonso WJ, et al. Global seasonality of rotavirus disease. Pediatr Infect Dis J 2013;**32**(4):e134-47.
318. Fouque F, Reeder JC. Impact of past and on-going changes on climate and weather on vector-borne diseases transmission: a look at the evidence. Infect Dis Poverty 2019;**8**(1):51.
319. Yalcin SS, Ozdemir B, Ozdemir S, et al. Agreement Between Integrated Management of Childhood Illness and Final Diagnosis in Acute Respiratory Tract Infections. Indian J Pediatr 2018;**85**(12):1086-89.
320. Minodier L, Masse S, Capai L, et al. Clinical and virological factors associated with gastrointestinal symptoms in patients with acute respiratory infection: a two-year prospective study in general practice medicine. BMC Infect Dis 2017;**17**(1):729.
321. Proenca-Modena JL, Gagliardi TB, Paula FE, et al. Detection of human bocavirus mRNA in respiratory secretions correlates with high viral load and concurrent diarrhea. PLoS One 2011;**6**(6):e21083.
322. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012;**379**(9832):2151-61.
323. Christopher PR, David KV, John SM, et al. Antibiotic therapy for Shigella dysentery. Cochrane Database Syst Rev 2010(8):CD006784.
324. Tickell KD, Brander RL, Atlas HE, et al. Identification and management of Shigella infection in children with diarrhoea: a systematic review and meta-analysis. Lancet Glob Health 2017;**5**(12):e1235-e48.

325. Tadesse BT, Ashley EA, Ongarello S, et al. Antimicrobial resistance in Africa: a systematic review. BMC Infect Dis 2017;**17**(1):616.
326. Roggen I, van Berlaer G, Gordts F, et al. Centor criteria in children in a paediatric emergency department: for what it is worth. BMJ Open 2013;**3**(4).
327. Musa-Aisien AS, Ibadin OM, Ukoh G, et al. Prevalence and antimicrobial sensitivity pattern in urinary tract infection in febrile under-5s at a children's emergency unit in Nigeria. Ann Trop Paediatr 2003;**23**(1):39-45.
328. Ibeneme CA, Oguonu T, Okafor HU, et al. Urinary tract infection in febrile under five children in Enugu, South Eastern Nigeria. Niger J Clin Pract 2014;**17**(5):624-8.
329. Masika WG, O'Meara WP, Holland TL, et al. Contribution of urinary tract infection to the burden of febrile illnesses in young children in rural Kenya. PLoS One 2017;**12**(3):e0174199.
330. Mitiku E, Amsalu A, Tadesse BT. Pediatric Urinary Tract Infection as a Cause of Outpatient Clinic Visits in Southern Ethiopia: A Cross Sectional Study. Ethiop J Health Sci 2018;**28**(2):187-96.
331. van de Sande-Bruinsma N, Grundmann H, Verloo D, et al. Antimicrobial drug use and resistance in Europe. Emerg Infect Dis 2008;**14**(11):1722-30.
332. Hopkins H, Bruxvoort KJ, Cairns ME, et al. Impact of introduction of rapid diagnostic tests for malaria on antibiotic prescribing: analysis of observational and randomised studies in public and private healthcare settings. BMJ 2017;**356**:j1054.
333. Nadjm B, Amos B, Mtove G, et al. WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: prospective study. BMJ 2010;**340**:c1350.
334. Batwala V, Magnussen P, Nuwaha F. Antibiotic use among patients with febrile illness in a low malaria endemicity setting in Uganda. Malar J 2011;**10**:377.
335. Johansson EW, Gething PW, Hildenwall H, et al. Effect of diagnostic testing on medicines used by febrile children less than five years in 12 malaria-endemic African countries: a mixed-methods study. Malar J 2015;**14**:194.
336. Baltzell K, Elfving K, Shakely D, et al. Febrile illness management in children under five years of age: a qualitative pilot study on primary health care workers' practices in Zanzibar. Malar J 2013;**12**:37.
337. Johansson EW, Kitutu FE, Mayora C, et al. It could be viral but you don't know, you have not diagnosed it: health worker challenges in managing non-malaria paediatric fevers in the low transmission area of Mbarara District, Uganda. Malar J 2016;**15**:197.
338. Rambaud-Althaus C, Shao AF, Kahama-Maro J, et al. Managing the Sick Child in the Era of Declining Malaria Transmission: Development of ALMANACH, an Electronic Algorithm for Appropriate Use of Antimicrobials. PLoS One 2015;**10**(7):e0127674.
339. Shao AF, Rambaud-Althaus C, Samaka J, et al. New Algorithm for Managing Childhood Illness Using Mobile Technology (ALMANACH): A Controlled

Non-Inferiority Study on Clinical Outcome and Antibiotic Use in Tanzania. PLoS One 2015;**10**(7):e0132316.
340. Ameyaw E, Nguah SB, Ansong D, et al. The outcome of a test-treat package versus routine outpatient care for Ghanaian children with fever: a pragmatic randomized control trial. Malar J 2014;**13**:461.
341. Baiden F, Webster J, Owusu-Agyei S, et al. Would rational use of antibiotics be compromised in the era of test-based management of malaria? Trop Med Int Health 2011;**16**(2):142-4.
342. Lanata CF, Rudan I, Boschi-Pinto C, et al. Methodological and quality issues in epidemiological studies of acute lower respiratory infections in children in developing countries. Int J Epidemiol 2004;**33**(6):1362-72.
343. Castro AV, Nascimento-Carvalho CM, Ney-Oliveria F, et al. Additional markers to refine the World Health Organization algorithm for diagnosis of pneumonia. Indian Pediatr 2005;**42**(8):773-81.
344. Puumalainen T, Quiambao B, Abucejo-Ladesma E, et al. Clinical case review: a method to improve identification of true clinical and radiographic pneumonia in children meeting the World Health Organization definition for pneumonia. BMC Infect Dis 2008;**8**:95.
345. Scott JA, Wonodi C, Moisi JC, et al. The definition of pneumonia, the assessment of severity, and clinical standardization in the Pneumonia Etiology Research for Child Health study. Clin Infect Dis 2012;**54 Suppl 2**:S109-16.
346. Fancourt N, Deloria Knoll M, Baggett HC, et al. Chest Radiograph Findings in Childhood Pneumonia Cases From the Multisite PERCH Study. Clin Infect Dis 2017;**64**(suppl_3):S262-S70.
347. Cutts FT, Zaman SM, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. Lancet 2005;**365**(9465):1139-46.
348. Berkley JA, Maitland K, Mwangi I, et al. Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study. BMJ 2005;**330**(7498):995.
349. Hamer DH, Brooks ET, Semrau K, et al. Quality and safety of integrated community case management of malaria using rapid diagnostic tests and pneumonia by community health workers. Pathog Glob Health 2012;**106**(1):32-9.
350. Alves Galvao MG, Rocha Crispino Santos MA, Alves da Cunha AJ. Antibiotics for preventing suppurative complications from undifferentiated acute respiratory infections in children under five years of age. Cochrane Database Syst Rev 2016;**2**:CD007880.
351. Agarwal G, Awasthi S, Kabra SK, et al. Three day versus five day treatment with amoxicillin for non-severe pneumonia in young children: a multicentre randomised controlled trial. BMJ 2004;**328**(7443):791.

352. Senn N, Rarau P, Salib M, et al. Use of antibiotics within the IMCI guidelines in outpatient settings in Papua New Guinean children: an observational and effectiveness study. PLoS One 2014;**9**(3):e90990.
353. Hazir T, Nisar YB, Abbasi S, et al. Comparison of oral amoxicillin with placebo for the treatment of world health organization-defined nonsevere pneumonia in children aged 2-59 months: a multicenter, double-blind, randomized, placebo-controlled trial in pakistan. Clin Infect Dis 2011;**52**(3):293-300.
354. McCollum ED, Brown SP, Nkwopara E, et al. Development of a prognostic risk score to aid antibiotic decision-making for children aged 2-59 months with World Health Organization fast breathing pneumonia in Malawi: An Innovative Treatments in Pneumonia (ITIP) secondary analysis. PLoS One 2019;**14**(6):e0214583.
355. King C, Colbourn T, Mankhambo L, et al. Non-treatment of children with community health worker-diagnosed fast-breathing pneumonia in rural Malawi: exploratory subanalysis of a prospective cohort study. BMJ Open 2016;**6**(11):e011636.
356. Higdon MM, Le T, O'Brien KL, et al. Association of C-Reactive Protein With Bacterial and Respiratory Syncytial Virus-Associated Pneumonia Among Children Aged <5 Years in the PERCH Study. Clin Infect Dis 2017;**64**(suppl_3):S378-S86.
357. Keitel K, Kagoro F, Samaka J, et al. A novel electronic algorithm using host biomarker point-of-care tests for the management of febrile illnesses in Tanzanian children (e-POCT): A randomized, controlled non-inferiority trial. PLoS Med 2017;**14**(10):e1002411.
358. Lubell Y, Blacksell SD, Dunachie S, et al. Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. BMC Infect Dis 2015;**15**:511.
359. Do NT, Ta NT, Tran NT, et al. Point-of-care C-reactive protein testing to reduce inappropriate use of antibiotics for non-severe acute respiratory infections in Vietnamese primary health care: a randomised controlled trial. Lancet Glob Health 2016;**4**(9):e633-41.
360. Erdman LK, D'Acremont V, Hayford K, et al. Biomarkers of Host Response Predict Primary End-Point Radiological Pneumonia in Tanzanian Children with Clinical Pneumonia: A Prospective Cohort Study. PLoS One 2015;**10**(9):e0137592.
361. Mahende C, Ngasala B, Lusingu J, et al. Profile of C-reactive protein, white cells and neutrophil populations in febrile children from rural north-eastern Tanzania. Pan Afr Med J 2017;**26**:51.
362. Hildenwall H, Muro F, Jansson J, et al. Point-of-care assessment of C-reactive protein and white blood cell count to identify bacterial aetiologies in malaria-negative paediatric fevers in Tanzania. Trop Med Int Health 2017;**22**(3):286-93.
363. Althaus T, Greer RC, Swe MMM, et al. Effect of point-of-care C-reactive protein testing on antibiotic prescription in febrile patients attending primary

care in Thailand and Myanmar: an open-label, randomised, controlled trial. Lancet Glob Health 2019;**7**(1):e119-e31.
364. Lubell Y, Althaus T. Biomarker tests for bacterial infection-a costly wait for the holy grail. Lancet Infect Dis 2017;**17**(4):369-70.
365. Peters RP, Twisk JW, van Agtmael MA, et al. The role of procalcitonin in a decision tree for prediction of bloodstream infection in febrile patients. Clin Microbiol Infect 2006;**12**(12):1207-13.
366. Bauch JA, Gu JJ, Msellem M, et al. Perception of malaria risk in a setting of reduced malaria transmission: a qualitative study in Zanzibar. Malar J 2013;**12**:75.
367. Ishengoma DS, Mmbando BP, Mandara CI, et al. Trends of Plasmodium falciparum prevalence in two communities of Muheza district North-eastern Tanzania: correlation between parasite prevalence, malaria interventions and rainfall in the context of re-emergence of malaria after two decades of progressively declining transmission. Malar J 2018;**17**(1):252.
368. Ghebreyesus TA, Admasu K. Countries must steer new response to turn the malaria tide. Lancet 2018;**392**(10161):2246-47.
369. Shah JA, Emina JB, Eckert E, et al. Prompt access to effective malaria treatment among children under five in sub-Saharan Africa: a multi-country analysis of national household survey data. Malar J 2015;**14**:329.
370. Lunze K, Biemba G, Lawrence JJ, et al. Clinical management of children with fever: a cross-sectional study of quality of care in rural Zambia. Bull World Health Organ 2017;**95**(5):333-42.
371. Mabey D, Peeling RW, Ustianowski A, et al. Diagnostics for the developing world. Nat Rev Microbiol 2004;**2**(3):231-40.
372. Delaigue S, Bonnardot L, Steichen O, et al. Seven years of telemedicine in Medecins Sans Frontieres demonstrate that offering direct specialist expertise in the frontline brings clinical and educational value. J Glob Health 2018;**8**(2):020414.
373. Keitel K, D'Acremont V. Electronic clinical decision algorithms for the integrated primary care management of febrile children in low-resource settings: review of existing tools. Clin Microbiol Infect 2018;**24**(8):845-55.
374. Decuypere S, Maltha J, Deborggraeve S, et al. Towards Improving Point-of-Care Diagnosis of Non-malaria Febrile Illness: A Metabolomics Approach. PLoS Negl Trop Dis 2016;**10**(3):e0004480.
375. Cordey S, Hartley MA, Keitel K, et al. Detection of novel astroviruses MLB1 and MLB2 in the sera of febrile Tanzanian children. Emerg Microbes Infect 2018;**7**(1):27.