Changing Etiologies of Febrile Illness in Areas of Differing Malaria Transmission in Rural Kenya

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Abstract

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Background: As malaria continues to decline in many parts of sub-Saharan African (SSA) and malaria rapid diagnostic tests become increasingly used; a growing number of febrile children are now being diagnosed as not having malaria. In order for these children to receive appropriate treatments, information on common non-malaria causes of fever, and treatment practices for febrile children without malaria is critically required to inform judicious use of antimalarials and antibiotics. At sites of high and low malaria endemicity in western Kenya; Kisii and Homa Bay, we conducted a 2-year cross-sectional surveillance study of febrile illness among children aged 6 months to 15 years to determine the: 1) frequency and correlates of malaria overtreatment and, 2) prevalence, causative organisms, and predictors of bacteremia due to any pathogen and specific bacterial pathogens among children seeking for fever.

Methods: Sociodemographic, environmental and clinical data were collected, and children tested for malaria, HIV, and bacteremia. Correlates of malaria overtreatment, and bacteremia were evaluated using multivariate logistic regressions.

Results: Nearly 7% of the 685 children enrolled in Kisii, and 45.8% of 677 Homa Bay had laboratory-confirmed malaria; p<0.001. Malaria overtreatment was more common in Homa Bay (57.2%), a high malaria endemic area with entomological infection rates (EIR) ≥300 than in Kisii (7.0%), a low malaria endemic area with EIR <1.5. Predictors of overtreatment in Homa Bay included presence of ≥1 Integrated Management of Childhood Illness (IMCI) danger signs (aOR=8.5; 95% CI: 4.8-14.9), fever lasting ≥7 days (aOR=4.9; 95%CI: 1.9-12.9), and fever ≥39^{0}C (aOR=3.1; 95%CI: 1.6-6.0). In Kisii, only fever ≥39^{0}C predicted overtreatment (aOR=2.1; 95%CI: 1.0-4.5). Malaria endemicity influences the clinical management of febrile children and may result in missed opportunities to treat alternative causes of fever. There is need to strengthen adherence to treatment guidelines to improve management of febrile children, to reduce risk of missed treatment opportunities for non-malaria fevers, particularly in malaria endemic areas.

The prevalence of bacteremia was 3.3% (48/1478); including 3.1% (24/734) in Kisii and 3.4% (24/742) in Homa Bay. *Salmonella spp*. (19 typhi and 21 nontyphoidal salmonella [NTS]) accounted for 83% of isolates, and did not differ between study sites. Bacteremia prevalence in children with and without malaria was 1.9% and 3.8% respectively (p=0.05). Co-infection with bacteremia and malaria was uncommon, <0.5%. Bacteremia was associated with incomplete vaccination (adjusted Odds Ratio [aOR]=2.1; 95% CI: 1.1-4.1), recent treatment with antimalarials (aOR=2.7; 95%CI: 1.4-4.1), having sought health-care elsewhere (aOR=2.2; 95% CI: 1.2-4.0) and lower education of caregiver (aOR=2.5; 95% CI: 1.1-4.8). NTS bacteremia was associated with HIV-infection (aOR=6.8; 95% CI: 1.2-38.8) and anemia (aOR=5.2; 95% CI: 1.4-18.9). Bacteremia was relatively uncommon and similar in both sites. However, children with HIV, with anemia, those who are incompletely vaccinated or those with persistent fever despite malaria treatment, may have higher risk and may benefit from targeted bacterial culture and/or empiric antibiotic therapy

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Table of Contents

List of Tables 9

List of Figures 10

Chapter 1: Introduction 11

Febrile illness among children in sub-Saharan Africa 11

Changing etiologies of febrile illness among children in sub-Saharan Africa 11

Rationale for this dissertation 12

Dissertation aims 13

Composition of the dissertation 13

Chapter 2: Background 14

Malaria over-diagnosis over-treatment in African children 14

Reasons for malaria over-diagnosis and overtreatment 14

Consequences of malaria over-treatment 16

Bacteremia as cause of febrile illness in African children 18

Spectrum of pathogens causing bacteremia among febrile African children 19

Chapter 3: Study settings, populations and methods 22

Study sites and settings 22

Study design, population and eligibility 23

Data storage and Management 25

Statistical analysis plan 26

Chapter 4: Frequency and Correlates of Malaria Over-treatment 27

Abstract 27

Background 29

Methods 30

Study Design and Population 30
Hospital clinical staff 31
Data Collection 31
Study Outcomes 32
Statistical Analysis 32
Results 33
Demographic, medical history, and clinical characteristics 33
Accuracy of the clinicians' diagnosis of malaria 36
Malaria treatment and correlates of malaria over-treatment 36
Discussion 40
Chapter 5: Bacteremia Among Febrile Children in Areas of Differing Malaria Transmission in Rural Kenya 44
Abstract 44
Background 46
Materials and Methods 47
Study sites and settings 47
Study design, population and eligibility 47
Clinical data collection 48
Laboratory data collection 48
Statistical Analysis 49
Results 50
Demographic, medical history, and clinical characteristics 50
Prevalence of bacteremia, and causative organisms 51
Correlates of bacteremia due to any pathogen 54
Correlates of bacteremia due to NTS and salmonella typhi 57

Discussion 58

Chapter 6: Discussion and conclusions 62

Malaria over-treatment 62

Community-acquired bacteremia 63

Practical and policy implications of the study 64

Further research or directions 64

List of Tables

Table 1: Study inclusion and exclusion criteria 23

Table 2: Primary study outcomes, exposures, confounders, and analysis techniques 26

Table 3: Demographic and clinical characteristics of study children 35

Table 4: Sensitivity and specificity of clinical diagnosis compared to 36

Table 5: Univariate and multivariate analysis of correlates of over-treatment in Kisii..... 37

Table 6: Univariate and multivariate analysis of correaltes of over-treatment in Homa Bay 38

Table 7: Demographic and clinical characteristics of study children and caregivers 52

Table 8: Prevalence of bacteremia and relative importance of causative agents..... 54

Table 9: Univariate and multivariate analyses of correlates of bacteremia 56

List of Figures

Figure 1: Recent studies of bacteremia among febrile Africa 19

Figure 2: Map of locations of study sites and underlying malaria and HIV prevalence 23

Figure 3: Recruitment into the malaria over-treatment study 34

Figure 4: Predictors of malaria over-treatment by site 39

Figure 5: Recruitment into the bacteremia study 52

Figure 6: Predictors of bacteremia infection among febrile children 57

Figure 7: Predictors of non-typhoidal salmonella (NTS) bacteremia 57

Chapter 1: Introduction

Febrile illness among children in sub-Saharan Africa

Febrile illness is a leading cause of morbidity and mortality among children in sub-Saharan Africa (SSA)[1]. For many years, fever in children of SSA was presumptively treated as malaria[2]. However, in the past decade, malaria has substantially declined in many endemic settings of SSA[3, 4], and most of the febrile illness is now due to viruses, and invasive bacteria, including previously under-recognized animal-associated bacterial bloodstream infections[5-8]. Some of these pathogens cause considerable mortality in children than malaria, even in endemic areas of Africa. For example, in rural settings of SSA, where malaria is common, bacteremia was responsible for more deaths in hospitalized children than malaria[9-11]. Mortality in febrile children diagnosed and treated for malaria when they actually have other pathogens is over 2-fold higher than in children who benefit from laboratory confirmed malaria[12]. Therefore, as malaria continues to decline in SSA, overtreatment of malaria in febrile children will result in even more increased failure to treat and prevent life-threatening non-malaria causes of fever and death.

Changing etiologies of febrile illness among children in sub-Saharan Africa

A growing body of evidence shows that invasive bacteria including previously under-recognized animal-associated bacteria, and viral infections have now replaced malaria as the most common cause of severe febrile illness among children in Africa, with substantial effects on morbidity and mortality[6, 7, 13, 14]. The relative importance of these pathogens to childhood febrile illness depends on local environment and prevalence of various risk factors such as HIV, malnutrition, anemia, environmental hygiene and sanitation, as well as vaccination coverage. Streptococcus pneumonia (S. pneumonia), nontyphoidal salmonella (NTS), haemophilus influenza (H. influenza), escherichia coli (E. coli), and staphylococcus aureus (S. Aureus) have consistently

been reported as the most frequent bacterial causes of severe febrile illness among African children and, both combined account for more than 70% of the blood culture isolates[9, 15, 16]. Additionally, animal-associated infections like leptospirosis, brucellosis, rickettsioses have also recently emerged as common causes of fever in African children with fatal consequences[6].

Recently, a study differentiating the bacterial, viral and parasitic causes fever in Tanzanian children found that viral infections were responsible for a large proportion (70%) of febrile illnesses in children presenting to hospitals, more than bacteremia and malaria infections combined, suggesting that treatment with antimalarials or antibiotics was not required in a majority of the children[7].

Rationale for this dissertation

As malaria continues to decline in many parts of sub-Saharan African[3, 8], and malaria rapid diagnostic tests increasingly become available and used to screen febrile children, there is an increasing recognition of the growing importance of non-malaria fevers to child morbidity and mortality in Africa[1]. However, studies of non-malaria causes of febrile illness in children in different countries and settings have shown wide geographical, and rural-urban differences in the relative importance of bacteremia and its associated etiologies to non-malaria febrile illness in children in SSA[6, 7, 17, 18]. Besides, as malaria increasingly becomes an uncommon cause of fever in children, health workers in rural settings may ignore negative malaria test results and treat febrile patients with antimalarials following many years' practice of treating fever as malaria. There is, therefore, need for locally relevant information regarding the contribution of bacteremia to non-malaria fever in children in SSA; and to evaluate clinicians' adherence to new WHO malaria treatment guidelines that emphasize restricting antimalarials to only laboratory confirmed cases of malaria. This information will be critical to informing evidence-based strategies for optimizing treatments for febrile illness in children, and decreasing inappropriate

use of antimalarial and antibiotic drugs.

We conducted a prospective cross-sectional surveillance study of febrile illnesses in children aged 6 months-15 years at two regional rural hospitals in Western Kenya; Kisii Provincial Hospital situated in an area of low malaria endemicity and Homa Bay Hospital situated in a malaria endemic area with the following objectives

Dissertation aims

i. To determine diagnostic accuracy clinicians' diagnosis of malaria in children compared laboratory testing, and the frequency and predictors of malaria over-treatment .
ii. To determine the prevalence, causative organisms and risk factors of community-acquired bacteremia.

Composition of the dissertation

This dissertation comprises a background covering changes in the causes of severe febrile illness, malaria diagnosis and treatment practices, and bacteremia as a cause of febrile illness in African children. The characteristics of the study sites and generic methods are presented as a single chapter, after which the main chapters are: chapter 3 where we present our findings of the frequency and correlates of malaria over-diagnosis and overtreatment, and chapter 4 in which we present findings about bacteremia among febrile children in areas of differing malaria transmission in rural Kenya. Each of these chapters includes a brief background, additional specific method statistical analyses, results and relevant discussion sections. The overall discussion and the conclusions are presented in chapter 6.

Chapter 2: Background

Malaria over-diagnosis over-treatment in African children

Malaria over-diagnosis refers to a diagnosis of malaria in a patient with no parasitemia[19]. This includes an incorrect presumptive diagnosis of malaria without laboratory confirmation or a diagnosis of malaria despite a negative laboratory test for malaria. Malaria overtreatment on the other hand, refers to a prescription of antimalarial drugs to a patient with negative a laboratory malaria test[12].

Depending on malaria transmission patterns in a given area, overwhelming evidence from across sub-Saharan Africa shows that a large proportion of children currently treated for malaria in endemic settings of Africa actually do not have malaria when tested[12, 20, 21]. In Tanzania, more than half of the children and adults patients with fever were presumptively diagnosed and treated with antimalarials, but only fewer than 2% actually had malaria when tested[6]. Overall, studies suggest that in some endemic regions up to 90% of all patients with fever are prescribed antimalarial drugs as their main treatment, but only very few have malaria when tested[21, 22]. As malaria continues to decline in most endemic settings of Africa, continued presumptive diagnosis and treatment will lead to even more massive over-diagnosis and over-treatment, and increased neglect of treating other causes of fever with fatal consequences.

Reasons for malaria over-diagnosis and overtreatment

The reasons for over-diagnosis of malaria are most likely multifactorial, and any attempts to improve malaria diagnosis and treatment practices will have to take this into account[23]. Malaria over-diagnosis and overtreatment among children has often been attributed to the wholesome implementation of WHO's Integrated Management of Childhood Illness (IMCI) guidelines that promoted presumptive treatment of fever as malaria for children living in malaria

endemic settings[2, 24]. These guidelines, which have since been reviewed to include malaria testing part of integrated management of febrile illness in children[25], supported a policy of testing for malaria in young children, but also allowed provision of antimalarial treatment regardless of the test result if clinicians suspected malaria. Clinicians also over-treat for malaria for several other reasons; the major one being the fear of missing out on possible true malaria cases with potentially fatal consequences. Because of the perceived increased risk of malaria mortality if treatment is missed, some clinicians consider it safe to treat several cases of non-malarial febrile illnesses with antimalarials than to miss a true case[26]. Current evidence, however, shows that withholding antimalarials from febrile children with negative malaria test results is clinically safe, even in for children in highly endemic areas[27-30].

Clinicians also over-treat for malaria because they don't trust test results. In Uganda, more than half of febrile children without malaria received antimalarial drugs, but only because clinicians did not trust the accuracy of the negative test results of malaria rapid diagnostic tests[30]. Whereas RDTs will miss some clinically uncomplicated malaria infections, studies show that these are mainly low-density and potentially self-limiting infections which rarely develop into severe malaria[28].

Chandler et al[31] studied in a qualitative study reasons for malaria over-treatment among clinicians, medical training tutors, and student clinical officers at two hospitals in Tanzania. Common reasons identified included the lack of laboratory diagnostic facilities, motivation and supervision, peer pressure and the need to conform to expectations of colleagues. Other reasons included a strong influence of teachings in medical training colleges, and national guidelines that overemphasized malaria as a rapidly progressive disease with mortality consequences if treatment for malaria is missed. Thus clinicians dreaded failing to treat malaria as soon as possible.

Patient or caregiver pressure and requests for antimalarial drugs have also been reported as important drivers of clinicians' decision to treat for malaria[32] . Afrane et al(2011)[20] found that patients in western Kenya often requested antimalarial drugs despite negative malaria tests because they strongly believed they needed them. In this study, most patients did not want to be tested for malaria before being treated presumptively because they did not see need for laboratory testing or they could not afford the cost of laboratory testing. In Uganda, clinicians' decision to treat for malaria despite a negative test was influenced by increased workload, lack of diagnostics for diagnosing alternative causes of fever and patients' high expectations to be informed about the cause of their illness in the presence of negative test[32, 33].

Consequences of malaria over-treatment

Over-diagnosis and subsequent over-treatment of malaria have important consequences; financially and in terms of morbidity and mortality. The most important consequence of malaria over-diagnosis and overtreatment is the failure to treat the actual non-malaria causes of fever resulting in severe disease and increased mortality[12, 19, 34, 35]. Studies in malaria endemic areas of Africa found that mortality in malaria-negative children treated with antimalarial drugs alone was 2-fold higher than in malaria-positive children, but only because of the failure to treat invasive bacterial infections[12, 19, 35]. In these studies, up to 60% of the patients with non-malaria febrile illness was not treated with antibiotics, and majority were later found to have a bacterial bloodstream infections[12].

Malaria over-diagnosis is also a public health problem because it results in overestimated malaria burden that in turn leads to misallocation of financial and human resources to manage the disease. Additionally, over-diagnosis leads to wastage of antimalaria drugs in resource-poor countries already dealing with drug shortages, and promotes the development of parasite

resistance to artemesinin based combination therapy (ACT) currently used as the effective first line treatment against malaria in Africa[21, 36]. The emergence of resistance ACTs is considered the greatest global threat to the future success of malaria control.

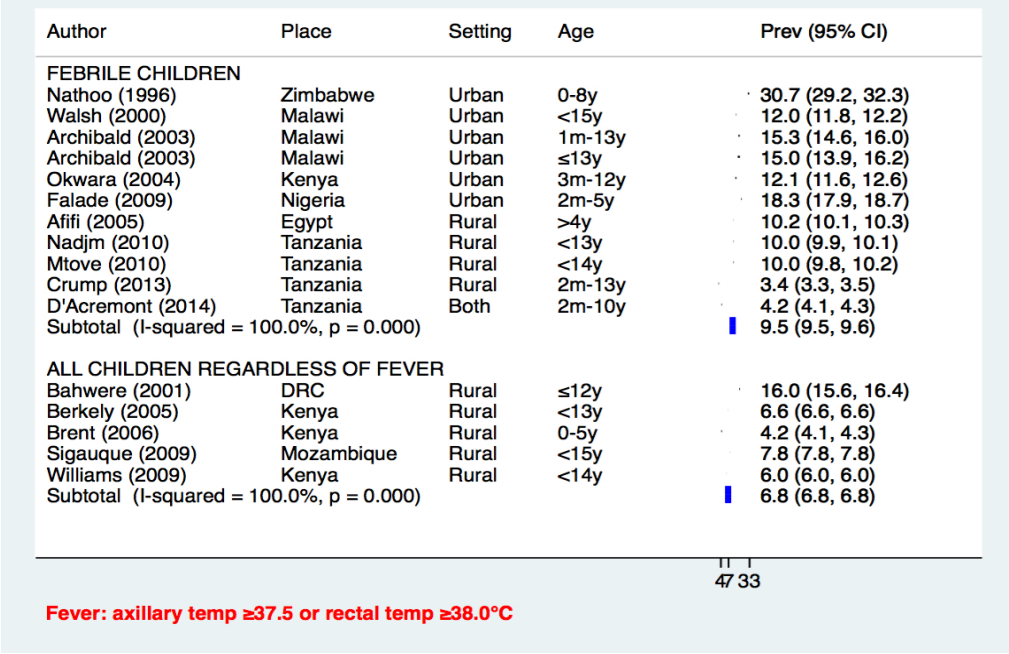
Financially, malaria over-diagnosis and over-treatment results in unnecessary healthcare visits, and associated increased treatment costs and reduced quality of care[37]. Over-treated patients often require subsequent consultations and additional treatments including more expensive drugs and even inpatient care. The result is increased treatment costs to households and health care providers[38]. A study in Malawi, found that low-income households spent over 30% of their annual income on direct (repeat visits and drugs), and indirect costs (transport, time off work) in seeking fever associated care. Indirect costs, such as transport and days off work for caregivers represented 79% of total costs of each fevers episode that were treated as malaria in Ghana[39]**.** Besides, lots of earnings are lost through prolonged ill health or time off work taken to look after sick children.

The economic effects of malaria over-diagnosis impact more heavily on the on the poor and vulnerable populations because they are less able to cope with the effects of prolonged ill health[21, 39] due untreated causes. Total health costs and lost earnings as a proportion of monthly expenditure owing to malaria over-diagnosis are also significantly higher among the poorest African households[37]. It has been estimated that if antimalarial drugs are prescribed only to patients with a positive diagnostic laboratory test, up to 60% of the costs of malaria treatment could be saved[40]. Finally, over-diagnosis and over-treatment exposes patients without malaria to unnecessary side effects of drugs and promotes the emergence of resistance to artemesinin-based combination therapys (ACTs).

Bacteremia as cause of febrile illness in African children

An accumulating body of evidence shows that febrile illnesses among African children are now caused by a wide variety of life-threatening invasive bacteria including previously unrecognized animal-associated bacterial pathogens[6, 41]. Population-based studies on the incidence of bacteremia among febrile African children with fever are scarce. These studies suggest that incidence rates per 100,000 person-years of bacteremia in febrile African children is as high as 1192[9, 15, 42, 43], and are even several-fold higher in children with underlying conditions such as HIV, and sickle-cell disease[44]. Sigauque and colleagues[45] estimated the incidence of bacteremia among febrile (fever ≥39^{0}C) African children under 15 years old living in a malaria endemic rural area of Mozambican that was under a continuous Demographic Surveillance System (DSS). The overall incidence of bacteremia was 425 per 100,000 child-years, including 1739 in children under 1 year, 782 in children ages 1-4 years old, and 49 in children 5 years and older. Bacteremia prevalences exceeding 13% among African children with fever has been reported[41]. Figure 1 below shows a summary of recent studies that have evaluated bacteremia as a cause of fever among African children. Although bacteremia tends to be more common among children with fever than those selected regardless of fever, there are significant geographical differences in the contribution of bacteremia to febrile illness in children as well as in the bacterial pathogens causing bacteremia[6, 7].

Figure 1: Recent studies of bacteremia among febrile Africa



Rates of bacteremia in African children vary widely, and largely depend on the population of children studied (sub-populations defined by age or temperature), risk factors for bacteremia at the study settings (e.g. prevalence of HIV, malnutrition, malaria)[41, 42, 46], place where the infection was acquired (community or in hospital), causative organisms[47], and the microbiological culture techniques used[48]. Among African HIV-infected children, rates of bacteremia are more than 20-fold higher, even among those on ART[49]. Overall, rates of bacteremia as a cause of severe febrile illness among African children are higher than those of malaria[11, 41, 42] underscoring the importance of bacteremia as a differential diagnosis of severe febrile illness in malaria endemic areas.

Spectrum of pathogens causing bacteremia among febrile African children

A wide range of treatable or preventable pathogens cause of fever in children of sub-Saharan African who present with malaria-like syndromes. The distribution of bacterial pathogens causing febrile illness in African children depends on several factors, including the geographical setting (urban or rural), specific environmental exposure related to rurality, patients' age,

household social economic status, vaccination status, co-morbidity (such as HIV-infection, malnutrition) and place of infection setting (community-acquired, healthcare associated or nosocomial)[18]. Pathogens such as streptococcus pneumonia (S. pneumoniae), non-typhoidal salmonella (NTS), haemophilus influenza (H. influenza), Escherichia coli (E. coli), and staphylococcus aureus (S. Aureus) have consistently been reported as the most frequent causes of febrile illness among African children, accounting for more than 70% of the blood culture isolates[9, 15, 16, 41]. Additionally, previously under-recognized animal-associated infections like leptospirosis, brucellosis, rickettsioses have recently emerged as important causes of fever in African children with substantial public health implications[6].

NTS bacteremia in febrile African children is highly associated with young age, severe and recent malaria, HIV, malnutrition, and possibly animal contact[18, 50]. S. pneumonia and S. Aureus bacteremias tend to be common among children with HIV, where S. pneumonia alone is responsible for more than a quarter of positive blood cultures[49]. In most of these pathogens, there seems to be differences between rural and urban areas in the rates and serotypes of bacteremia isolates[17, 18]. For example, in Kenya, rates of NTS bacteremia are very high in rural than urban children of the same age. On the contrary, rates of bacteremia due to S. typhi are much lower among Kenyan children residing in rural areas compared children of the same age who reside in urban areas[17]. These rural-urban differences in the rates of NTS and typhi have been attributed to local levels of poverty, overcrowding and lack of hygiene (e.g. Salmonella enterica), close contact with animals (e.g. NTS), as well as differences in prevalences of malaria, HIV and malnutrition. In Tanzania, NTS is common in areas with intense malaria transmission, while Salmonella typhi was common in areas with low malaria transmission[51]. There is therefore need for knowledge on locally relevant pathogens to avoid mismatches in antibiotic choices for patients presenting with severe infections, which might eventually impact on patient outcomes.

Bacteremia-related mortality in African children

Accumulating evidence shows that bacteremia causes substantial mortality among children of sub-Sahara Africa[9, 52-55], accounting for nearly 30% of all the childhood deaths in and outside of hospital. Mortality rate among children with bacteremia exceeds 40%[9, 54, 55] and it could be even higher due to non-ascertainment of additional mortality in febrile children that occur outside of hospital[9, 15]. Deaths in children with bacteremia occur rapidly, with up to 70% of these deaths occurring within the first two-days of admission to hospital[9, 19, 54]. This is equivalent to a 48-hour case fatality of about 17%, underscoring the need for early identification of the affected children for targeted treatment. Even in rural areas with high rates of HIV and malaria infections, there appears to be more deaths in children associated with bacteremia than with malaria[9, 10, 19] suggesting that bacteremia may be even more important as a cause of death than malaria among children in sub-Saharan Africa[11]. Overall, the clinical outcome of bacteremia infection among African children depends on causative pathogen characteristics (i.e. virulence, number of infecting pathogens, antibiotic resistance, hospital-acquired or community-acquired etc) and host factors (HIV, malnutrition, ages, male gender, inflammatory response) and receipt of prompt and appropriate antibiotic therapy[56]. .

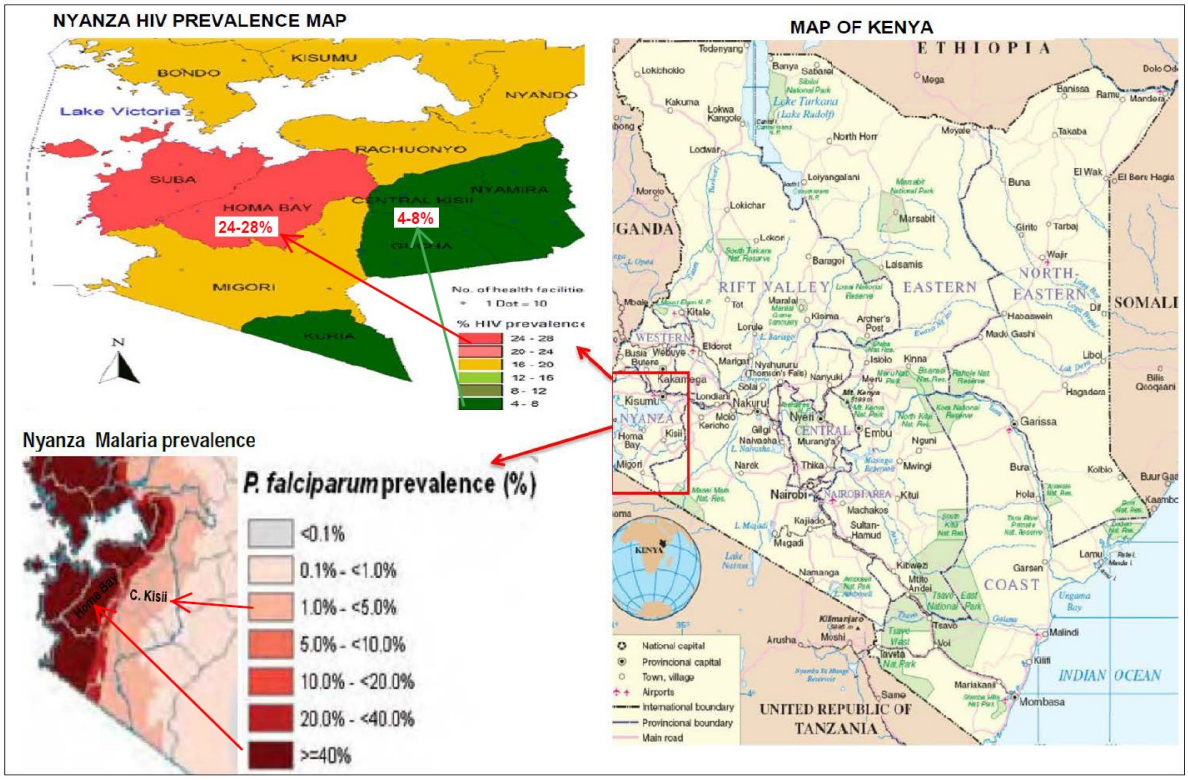
Chapter 3: Study settings, populations and methods

Study sites and settings

This dissertation research study was conducted at Kisii Provincial Hospital, and Homa Bay District Hospital both in rural Western Kenya. These hospitals serve rural regions that are geographically and demographically heterogeneous with significantly different rates of malaria transmission, HIV and malnutrition and poverty levels (Figure 2). Kisii Hospital is a regional primary and referral hospital with a 450-bed capacity, and serves a rural population of more than three million people, mainly mixed subsistence farmers. Malaria transmission in Kisii is low and seasonal with annual entomological inoculation rate (EIR) of ≤1.5 infectious mosquito bites per person per year[57, 58], with two seasonal peaks that coincide with two main rainy seasons spanning March to May, and October to November. The prevalence of HIV-infection among antenatal clients in Kisii ranges from 4-8%[59].

Homa Bay District Hospital, is a local primary and referral hospital serving a rural poor population of more than two million people, mainly substance farmers and fishermen. Compared to Kisii, Homa Bay region has intense and perennial malaria transmission[60], with an entomological inoculation rate as high as 300 infectious mosquito bites per person per year[61], and has HIV prevalence among antenatal clients is as high as 28%, widespread malnutrition and, poverty and low vaccine coverage[62-64]. Both Kisii and Homa Bay Hospitals provide primary care and are co-coordinating and first point of referral for the severely ill from the surrounding peripheral health facilities.

Figure 2: Map of locations of study sites and underlying malaria and HIV prevalence



Study design, population and eligibility

This was a multi-site cross-sectional surveillance study of pediatric febrile illnesses. Over a two-year period (March 2012 to March 2014), consecutive children ages 6 months to 15 years presenting with fever (axillary temperature ≥37.5^{0}C) were approached for enrolment. Details of the inclusion and exclusion are summarized Table 1 below.

Table 1: Study inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
• 6 months through 15 years of age • Presenting with fever (axillary temperature ≥ 37.5°C) • Not used antibiotics in last 24 hours • Parent or guardian willing to provide informed consent • Parent or guardian willing to attend counseling for DCT • Able to provide blood samples	• Use of antibiotics within the last 24 hours (did not include chronic suppressive antibiotic therapy such as septric for HIV or pencillin for chronic cardiovascular conditions • Unaccompanied by parent/ guardian

Clinical data collection

Dedicated study clinical officers or nurses administered an extensive standardized case report forms to record sociodemographics, presenting clinical signs and symptoms, physical examination findings, point-of-care laboratory test results, and environmental sanitation and hygiene information. Medical history, including prior antibiotic and antimalarial treatment in the week preceding hospitalization were also recorded. After consent was obtained, children underwent physical and clinical examination, and blood was drawn from consenting children by study phlebotomist for complete blood count, malaria, HIV, and blood culture testing. For children who were subsequently admitted, blood samples for culture were obtained within the first 48 hours of hospital admission. All children, except emergencies, were assessed using Integrated Management of Childhood Illness (IMCI) guidelines[65]. Children with suspected bacteremia, received immediate empiric treatment with antibiotics according to the guidelines (called Basic pediatric protocols) of the Kenya Ministry of Health (MOH)[66].

Details of specific laboratory tests including, HIV and malaria testing, and blood cultures are presented under each study aim in successful chapters. Briefly, children were tested for HIV per Kenya Ministry of Health guidelines. Blood specimens were collected using standard BACTEC Peds blood cultures bottles and shipped to the nearby US-Army Microbiology laboratory within 24 hours of collection where they were evaluated for pathogens. Pathogenic bacterial isolates were identified and evaluated for antibiotic susceptibilities using a Microscan Walkaway40® system.

Consenting

At admission, the study was explained to the child's caregiver in the national language Kiswahili, or local languages (Luo, Kisii etc), or English depending on a parent's preferred language. Caregivers were allowed time to read the consent forms, ask questions and consider their

decisions. The consent was read aloud to illiterate parents who were unable for read themselves. The study nurses or counselors were trained do this in a balanced manner to avoid possible coercion. Caregivers who agreed their children to participate in the study gave witnessed written and signed informed consent before study enrolment. Iliterate caregivers gave a witnessed thumbprint before enrolment. A copy of the signed consent form was given to the enrolled children's caregivers. The study protocol and related documents were all reviewed and approved by the research ethics committees of the University of Washington and the Kenya Medical Research Institute (KEMRI).

Data storage and Management

Data on socio-demographics, physical examination, laboratory results and prescribed treatments was entered into Daciforms, a comprehensive and secure web-based data management platform (Dacima Software, Inc., Montreal, QC). Within Dacifoms platform, clinical and laboratory data were uniquely linked using a PID number and stored in an encrypted format. All soft and hard copy data formats were kept in locked cabinets with limited access by project staff only.

Data verification and quality assurance

Before a patient left the clinic, the study clinical officers or nurses went through case report forms to ensure proper completion, and no inconsistent responses. At the central data office, the principal investigator assisted with a data manager extracted data from Daciforms database on an on-going basis to identify erroneous and missing data. Using data cleaning syntaxes coded in STATA (Stata Corp, College Station, TX, USA), the principal investigator searched for inconsistent entries in the databases and had them corrected as soon as possible using clinical records. On a weekly basis, the principal investigator cross-checked the data entered into Daciforms by generating detailed weekly reports to check for inconsistencies, inaccuracies and

missing data. These reports were discussed during weekly data meetings with the study clinical and laboratory teams. No personal identifiers were stored in the database and the only identifying information was on hard copy patient locator and consent forms that were stored in a locked file cabinets. Only study clinicians and the primary investigator accessed these cabinets.

Statistical analysis plan

The table below summarizes outcomes, primary exposures (correlates), a priori selected potential confounders, and analysis techniques for each study aim. Details of the implementation of these analyses will be provided under specific chapters about results for each aim.

Table 2: Primary study outcomes, exposures, confounders, and analysis techniques

Aims	Outcome	Primary Exposures	A *priori* confounders	Analysis technique
Aim 1: To determine the frequency and correlates of malaria over-treatment among Kenyan children age 6 months to 15 years who sought care for acute febrile illness.				
Aim 1	Malaria over-treatment	IMCI danger signs, fever >39°C, malnutrition, child HIV-infected, Mother HIV-infected, Fever for ≥7 days, sought care elsewhere, taken antimalarials or antibiotics last 7d,	Age, sex, inpatient vs. outpatient, caregiver's education, income,	Univariate and Multivariate logistic regression
	Accuracy of clinicians' diagnosis of malaria	N/A	Malaria endemicity (ssstratification variable)	Estimation of Sensitivity & Specificity of
Aim 2: To determine prevalence, etiologies and risk factors of community-acquired bacteremia among Kenyan children age 6 months to 15 years who sought care for acute febrile illness.				
Aim 2	Community-acquired bacteremia (CAB)	HIV, malnutrition, IMCI danger signs, exposure to animals, unsafe water, incomplete vaccination, fever >39°C, Fever for >=7 days, use of antimalarials or antibiotics last 7d, sought care elsewhere, malaria infection, caregiver's education, income.	Age, sex, mother's HIV	Univariate and Multivariate logistic regression

Chapter 4: Frequency and Correlates of Malaria Over-treatment

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Abstract

Background: Although WHO recommends laboratory confirmation of malaria before treatment, febrile children are frequently treated empirically. We evaluated accuracy of diagnosis and determined frequency and predictors of malaria over-treatment among febrile laboratory-confirmed malaria-negative children in Kenya.

Methods: We enrolled 1362 febrile children presenting to Kisii (n=685) and Homa Bay (n=677) hospitals over a 22-month period. Children were screened for HIV, for malaria using smear microscopy and rapid diagnostic tests and for bacteremia. We determined sensitivity and specificity of clinical malaria diagnosis and the frequency of antimalarial treatment among malaria-negative children. Predictors of over-treatment were identified using logistic regression. Because of differences in malaria endemicity, analyses were stratified and compared by site.

Results: Nearly 7% of the children in Kisii, and 45.8% in Homa Bay had laboratory-confirmed malaria; p<0.001. The sensitivity of clinical diagnosis of malaria was lower in Kisii than Homa Bay (73.0% vs. 95.3%; p<0.001) but specificity was higher in Kisii than Homa Bay (93.2% vs. 33.7%; p<001). Among malaria-negative children; 57.2% in Homa Bay and 7.0% in Kisii received antimalarials; p<0.001. Predictors of over-treatment in Homa Bay included ≥1 IMCI danger signs (aOR=8.5; 95% CI: 4.8-14.9), fever lasting ≥7 days (aOR=4.9; 95%CI: 1.9-12.9), and fever ≥39^0C (aOR=3.1; 95%CI: 1.6-6.0). In Kisii, only fever ≥39^0C predicted over-treatment (aOR=2.13; 95%CI: 1.0-4.5).

Conclusion: Despite availability of diagnostic testing, malaria prevalence appears to influence the clinical management of febrile children and may result in failure to manage alternative causes of fever. Strengthening adherence to treatment guidelines appears necessary, particularly in endemic areas.

Background

In sub-Saharan Africa, fever is one of the most common presenting signs in children seeking care, accounting for more than 30-50% of all pediatric hospital visits[67]. Because of the high malaria prevalence, coupled with high rates of malaria-attributable mortality, guidelines for fever management in children have historically promoted presumptive treatment of malaria in febrile children in endemic areas[2, 65, 68, 69]. However, fever may be due to a variety of other bacterial, viral and parasitic infections[8, 20, 25]. In addition, the proportion of febrile illness due to malaria has declined considerably in many African settings, even in highly endemic areas[8, 20, 25]. In Western Kenya, malaria transmission has declined by more than half since 2003 and it is now estimated over 80% of all fevers in Kenyan children are caused by other pathogens[4, 70]. Empiric treatment of febrile children with antimalarials often results in significant malaria over-treatment, which may result in the failure to treat other serious causes of fever, particularly bloodstream infections[12, 19, 20, 34, 35]. Mortality among children treated for malaria is over 2-fold higher in malaria-negative children than in children with laboratory confirmed malaria, often as a result of untreated bacterial infections[12, 19, 35]. Additionally, malaria over-treatment can lead to the emergence of drug resistance, unnecessary adverse drug effects, increased treatment costs, and reduced quality of care[21, 36].

Following considerable declines in malarial transmission in many parts of Africa, including in highly endemic areas; the increased recognition of the importance of life-threatening non-malaria fevers, and the wide availability of cheap and highly sensitive rapid diagnostic tests (RDTs), the World Health Organization (WHO) released new malaria treatment guidelines in 2010[71]. These guidelines emphasize laboratory testing of all suspected malaria cases and treatment of only laboratory confirmed malaria with antimalarials. While the Kenyan Ministry of Health has adopted these new guidelines and has made point-of-care malaria rapid diagnostic tests available to many health facilities in Kenya, fever continues to be treated empirically as

malaria in many settings, even when laboratory testing is negative[17, 58, 72]. Factors contributing to the continued malaria over-treatment are likely multi-factorial, including the longstanding practice of treating fevers as malaria, the inflated perception of local malaria risk by clinicians, and the low confidence in a negative laboratory test by clinicians[17]. Structural barriers, particularly in rural settings, may also contribute to the failure to follow recommended practice. Specifically, diagnostics may not be consistently available, knowledge of new malaria treatment guidelines among nurses and clinicians may be limited and systems to monitor and evaluate malaria diagnosis and treatment practices may be lacking[21].

This study evaluated malaria treatment practices at two regional rural hospitals in Kenya in order to understand the frequency and factors associated with malaria over-treatment in febrile children. The stimulus for this study was the observation that most febrile children presenting to the study sites were being treated for malaria even when test results were negative. By understanding modifiable correlates of malaria over-treatment, programs can reduce over-treatment, increase the diagnosis and management of alternative causes of fever and improve outcomes in febrile children in malaria endemic areas.

Methods

Study Design and Population

This was a cross-sectional study within an ongoing surveillance study of febrile illnesses among children ages 6 months to 15 years presenting with fever (axillary temperature ≥37.5^{0}C) at Kisii Provincial Hospital and Homa Bay District Hospital, both in Western Kenya. These hospitals are situated in areas with significantly different malaria endemicity; Kisii is hypoendemic with annual entomologic inoculation rate (EIR) of <1.5 infectious bites per person per year, while Homa Bay is holoendemic with EIR of 300 infectious bites per person per year[17, 58] . We excluded children unaccompanied by their parents or guardians, or whose parents did not consent to HIV

counseling and study participation on behalf of their children. This study was approved by ethics committees of the University of Washington and the Kenya Medical Research Institute.

Hospital clinical staff

As is common in Sub-Saharan Africa, clinical staff at the study sites primarily consists of trained clinical officers or registered nurses with three years of medical training and licensed to independently diagnose and treat diseases, including ordering and interpreting laboratory tests. There are very few medical doctors available and these specialists usually only handle complicated cases and administrative duties of the hospital.

Data Collection

Within the ongoing parent study, we systematically captured comprehensive information regarding socio-demographics, anthropometric characteristics, environmental sanitation and hygiene, past medical history and care seeking behavior, current clinical and laboratory data using standardized case report forms. Malaria was diagnosed using smear microscopy and Paracheck Pf® rapid diagnostic tests (Orchid Biomedical Services, India). HIV was determined using rapid antibody testing (Abbott Determine™ rapid test kit and confirmed using Uni-Gold™) or PCR if child ≤18 months. Blood was collected from all HIV-infected children for CD4 count/percent testing. Finally, blood for culturing was collected using BACTEC bottles and shipped to the US-Army Walter Reed Microbiology laboratory within 24 hours of collection. Positive cultures were gram stained and appropriately sub-cultured onto sheep blood, chocolate and MacConkey agar, and incubated for 24-48 hours to obtain bacterial colonies. Isolated colonies were identified and antibiotic susceptibility determined using a Microscan Walkaway40® system.

Study Outcomes

Laboratory confirmed malaria was defined as a positive smear microscopy and/or RDT test. Malaria over-treatment was defined as a negative smear and RDT result that was treated with antimalarials. Other outcomes included the accuracy of the clinicians' diagnosis of malaria when compared to the laboratory tests.

Statistical Analysis

Demographic, clinical and laboratory characteristics were compared between study sites using chi-square or Fisher's exact tests for categorical variables and two sample t-tests or Wilcoxon rank sum tests for continuous variables. We determined the proportion of febrile children with a positive laboratory malaria test among all children diagnosed and treated for malaria. We estimated the proportion of children who had negative blood smear and RDT but were treated with antimalarials and compared them between study sites using chi-square test. We used logistic regression models to identify associations between the primary outcome of malaria over-treatment and patients' clinical and demographic characteristics. To identify independent correlates of malaria over-treatment, we simultaneously included all the following a priori selected confounders in a multivariate logistic model: child's age, sex, whether the child was enrolled from out- or in-patient departments, primary caregiver's level of education and income. The latter two variables were used as surrogates for family socioeconomic status. Using combined laboratory tests as gold standard, we evaluated clinicians' diagnostic accuracy of malaria using sensitivity and specificity. We used Cohen's reliability kappa coefficient to assess concordance between the clinician's diagnosis and the laboratory diagnosis of malaria. Because the sites historically have marked differences in underlying malaria endemicities that might impact clinician suspicion of parasitemia, analyses were stratified and compared by site. All analyses were done using Stata statistical software (version 13.1, Stata Corp., College Station, TX).

Results

Demographic, medical history, and clinical characteristics

Between April 2012 and November 2013, 1462 febrile children were enrolled into the parent study; 685 in Kisii and 677 in Homa Bay. Figure 3 summarizes screening, enrolment, clinical treatment of malaria and, laboratory testing for malaria and blood cultures of study participants. Baseline demographic and clinical information of the enrolled children, stratified by enrollment site, are shown in Table 2. Compared to children from Kisii, children from Homa Bay were younger, more likely to be female, more likely to be acutely malnourished (wasted) and sicker at presentation as defined by the presence of at least one IMCI danger sign (52.6% vs. 16.1% $p<0.001$). In addition, as compared with children from Kisii, children in Homa Bay were more likely to have mothers infected with HIV (19.5% vs. 3.3%; $p<0.001$) or to be HIV infected themselves (4.5% vs. 1.3% $p<0.001$). However, children from Homa Bay were less likely to have previously sought healthcare elsewhere for their current illness than children from Kisii (23.0% vs. 33.9%, $p=0.001$).

Malaria Diagnoses

Among the 1462 febrile children enrolled, a presumptive clinical diagnosis of malaria was made and treatment offered in 44.3%. This differed significantly between Homa Bay (75.8%; 513/677) and Kisii (13.1%; 90/685). However, only 26% of all the children enrolled in the study (45.8% in Homa Bay and 6.7% in Kisii; Prevalence Ratio=6.8; 95CI: 5.2-8.9) were malaria-positive by RDT or smear. Among all 356 laboratory confirmed malaria cases, most (96.1%) were positive by both RDT and smear, 10 (2.8%) were positive by RDT alone and 4 (1.1%) were positive by smear alone. There were no significant differences in the proportion of malaria tests positive by only RDT or smear between the sites.

Figure 3: Recruitment into the malaria over-treatment study

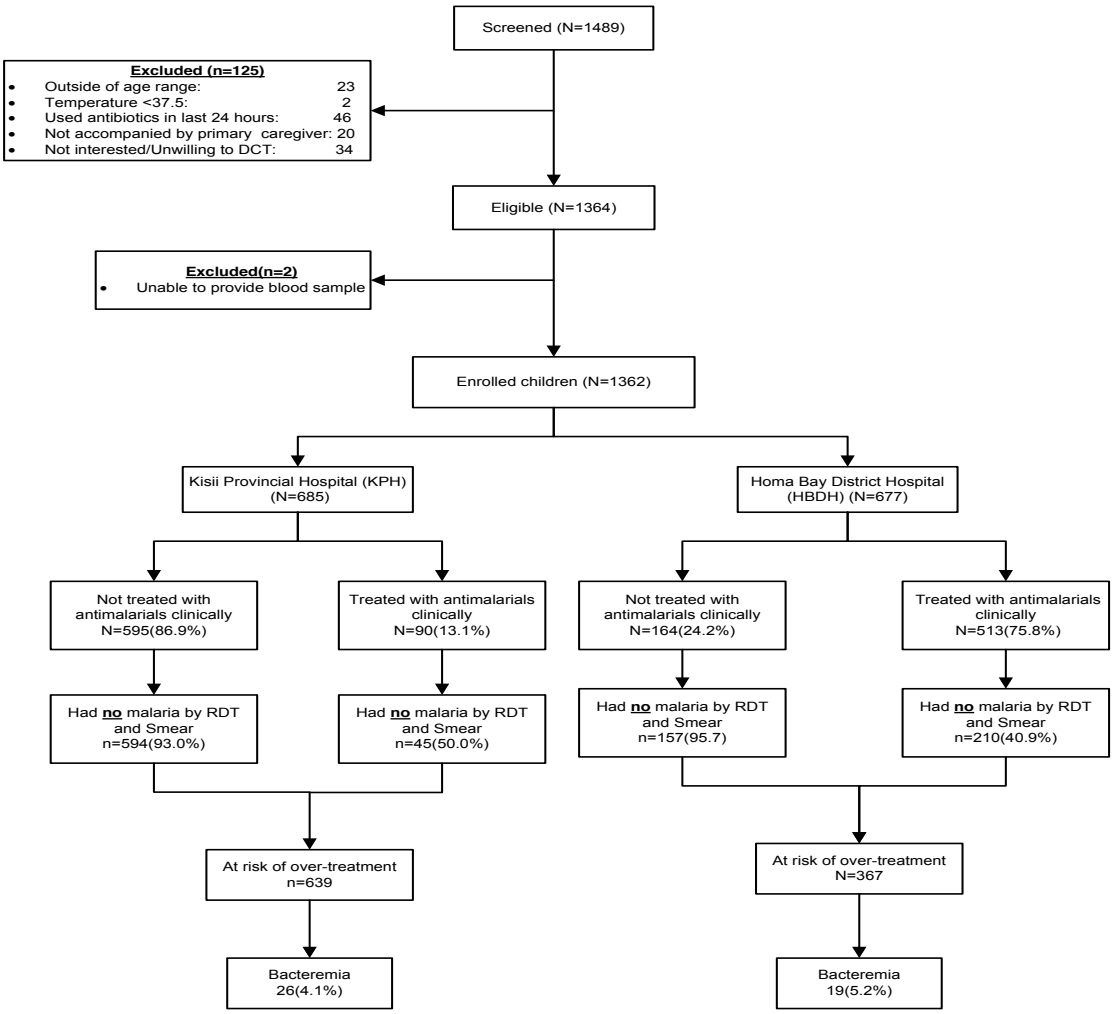


Table 3: Demographic and clinical characteristics of study children

Variable	Study Site				p-value
	Kisii (n=685)		Homa Bay (n=677)		
	n	(%)	n	(%)	
Demographics					
Child's sex: Female	306	(44.7)	346	(51.1)	0.017
Age in Years (Mean±SD)(Median)	3.1±2.1(2.9)		2.8±1.6(2.8)		0.012
Age(years)					<0.001
≤2yr	256	(37.4)	245	(36.2)	
3-4yr	335	(49.0)	386	(57.1)	
≥5yr	93	(13.6)	45	(6.7)	
Consumes water from unimproved sources[§]	26	(3.8)	145	(21.4)	<0.001
Primary caregiver's characteristics					
Education					<0.001
College/University	101	(14.8)	58	(8.6)	
Secondary	273	(39.9)	152	(22.5)	
At most Primary	310	(45.3)	467	(69.0)	
Income					<0.001
≤$50	189	(27.6)	361	(53.3)	
$50-100	275	(40.2)	220	(32.5)	
>$100	220	(32.2)	96	(14.2)	
Children's clinical presentations					
Malnutrition					
MUAC<12.5	15	(2.2)	25	(3.7)	0.087
Wasting (WHZ<-2)	115	(19.5)	78	(12.4)	0.001
Stunted (HAZ<-2)	83	(13.6)	98	(15.4)	0.272
Underweight (WAZ<-2)	52	(8.5)	75	(11.8)	0.044
Temperature(°C)					<0.001
37.5-37.9	138	(20.1)	67	(9.9)	
38.0-38.9	308	(45.0)	318	(47.0)	
39.0-39.9	202	(29.5)	239	(35.3)	
>=40.0	37	(5.4)	53	(7.8)	
High grade fever (>39°C)	206	(30.1)	262	(38.7)	0.001
Any IMCI general danger sign(s) present[†]	110	(16.1)	356	(52.6)	<0.001
Unable to drink/breastfeed	14		184		
Vomits everything	62		209		
Convulsions	30		51		
Lethargic/unconscious	43		70		
Past clinical history					
Has had fever lasting ≥7 days	263	(38.4)	79	(11.8)	<0.001
Sought healthcare elsewhere	232	(33.9)	156	(23.0)	<0.001
Taken antimalarials last 7days	86	(12.6)	106	(15.7)	0.100
Taken antibiotics last 7days	52	(7.6)	32	(4.7)	0.024
Laboratory results					
Mother HIV -infected	22	(3.3)	129	(19.5)	<0.001
Child HIV-infected	9	(1.3)	30	(4.5)	0.001
Malaria (smear or RDT)	46	(6.7)	310	(45.8)	<0.001
Pathogenic bloodstream bacteria[#]	27	(3.9)	25	(3.7)	0.811

[§]Unpiped water, unprotected boreholes, wells and springs, water from vendors. [†]Unable to feed and drink, vomits everything, convulsion, difficult to awaken or abnormally sleepy,

Accuracy of the clinicians' diagnosis of malaria

The sensitivity of a clinical diagnosis of malaria (as compared to laboratory confirmed malaria infection) was significantly lower in Kisii (73.0%; 95%CI: 55.9%-86.2%) than in Homa Bay (95.3%; 95%CI: 91.5%-97.7%) (Table 3). However, the specificity of clinicians' malaria diagnosis was higher in Kisii (93.2%; 95%CI: 90.5%-95.2%) than in Homa Bay (33.7%; 95%CI: 8.2%-39.6%), consistent with the level of over-treatment observed in Homa Bay. The estimated concordance between clinic diagnosis, and the laboratory results was 62.1% in Kisii and 34.4%% in Homa Bay.

Table 4: Sensitivity and specificity of clinical diagnosis compared to gold-standard laboratory diagnosis of malaria by site

	Site of Enrolment		
	Kisii	Homa Bay	
	Test % (95% CI)	Test % (95% CI)	p-value
Sensitivity	73.0%(55.9% - 86.2%)	95.3%(92.0% - 97.6%)	<0.001
Specificity	93.2%(90.5% - 95.2%)	33.7%(8.2% - 39.6%)	<0.001

Malaria treatment and correlates of malaria over-treatment

All children with laboratory confirmed malaria (n=356) were treated with antimalarials. Most received artemisinin combination therapy (ACT) (82.3%) while fewer received quinine (17.7%). Of those treated with quinine; 82.5% had high-grade fever or manifested IMCI danger signs. However, among children with both a negative blood smear and RDT test result (639 in Kisii and 367 in Homa Bay), the proportion of those inappropriately treated with antimalarials was significantly higher in Homa Bay than in Kisii (57.2% vs. 7.0%; p<0.001).

In Kisii, odds of malaria over-treatment were significantly higher for children with high-grade fever (Odds Ratio(OR)=2.1; 95%CI: 1.2-4.4), those who reported having taken antimalarials in the week preceding visit to hospital (OR=2.1; 95% CI: 1.0-4.4) and those whose mothers were

HIV-infected (OR=3.3; 95% CI: 1.1-9.6) in bivariate analysis (Table 4). But only the association of high-grade fever with malaria over-treatment persisted in the multivariate analysis (aOR=2.1; 95%CI: 1.0-4.5).

Table 5: Univariate and multivariate analysis of correlates of over-treatment in Kisii

	Over-treated				Crude	Adjusted[§]
Variable	**No (n=594)**		**Yes(n=45)**		**cOR(95% CI)**	**aOR(95% CI)**
Potential correlates	**n**	**(%)**	**n**	**(%)**		
Malnutrition indicators						
MUAC<12.5	13	(2.2)	1	(2.2)	1.0 (0.13-7.9)	1.6 (0.2-13.4)
Wasting	107	(20.8)	5	(14.3)	0.6 (0.24-1.7)	0.7 (0.2-1.9)
Stunted	73	(13.7)	3	(8.6)	0.6 (0.18-2.0)	0.8 (0.2-3.0)
Underweight	45	(8.4)	2	(5.7)	0.7 (0.15-2.8)	1.1 (0.12-7.1)
Mother HIV-infected	17	(3.0)	4	(9.1)	3.3(1.11-9.6)	1.8 (0.4-8.4)
Child HIV-infected	7	(1.2)	2	(4.5)	3.9 (0.9-17.3)	NA[†]
Any IMCI danger sign?	90	(15.2)	8	(17.8)	1.2 (0.6-2.7)	1.9 (0.7-5.2)
High grade fever (>39°C)	166	(27.9)	21	(46.7)	2.3 (1.2-4.2)	2.1 (1.0 - 4.5)
Fever for 7 or more days	232	(39.1)	19	(42.2)	1.1 (0.6-2.1)	1.5 (0.7-3.2)
Sought care elsewhere	195	(32.8)	14	(31.1)	0.9 (0.5-1.8)	0.8 (0.3-2.1)
Taken Antimalarial last 7d	70	(11.8)	10	(22.2)	2.1 (1.0-4.4)	2.5 (0.8-7.3)
Taken Antibiotics last 7d	47	(7.9)	2	(4.4)	0.5 (0.1-2.3)	0.3 (0.1-2.8)

[†]Data insufficient to include this variable in multivariate model only 2 of the children over-diagnosed in. Kisii were HIV+. [§]Adjusted for child's age, sex, and caregivers' education and

In Homa Bay, signs and symptoms of severe sickness were associated with over-treatment. The unadjusted odds of malaria over-treatment for children with both a negative blood smear and RTD test result were significantly higher for children with high-grade fever (OR=2.1 95%CI: 1.3-3.4), a history of fever lasting a week or more (OR=2.9; 95%CI: 1.4-6.1), and at least one IMCI danger signs present (OR=5.8; 95%CI: 3.7-9.1)(Table 5). However, the odds of over-treatment were lower for children who reported to have sought care prior to current hospital visit (OR=0.5; 95%CI: 0.3-0.8) and lower for children who had reported having taken antibiotics within the last one week (OR=0.4; 95%CI: 0.2-0.9). These associations persisted and strengthened in multivariate logistic regression analyses adjusting simultaneously for a priori selected confounders that included sex, age of the children, whether children were enrolled from

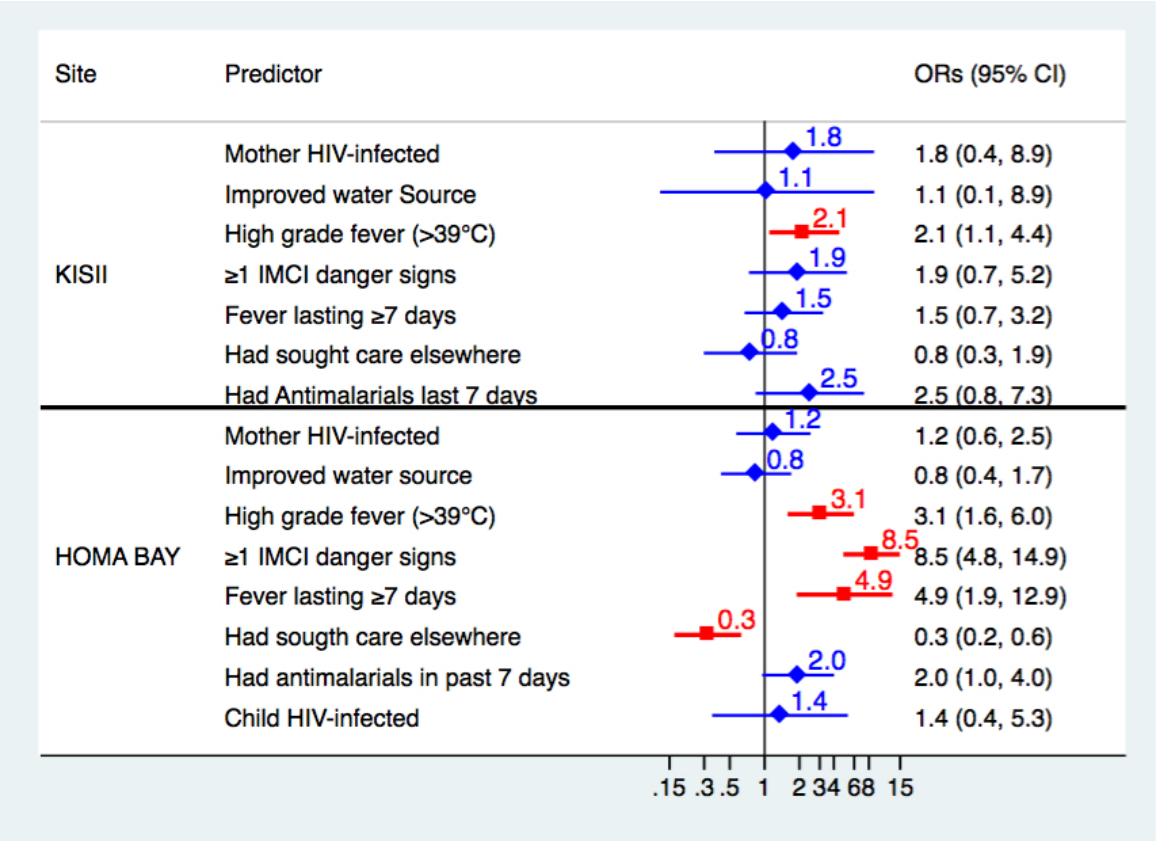
in-patient or outpatient department and primary caregivers' education and income. In multivariate analysis, malaria over-treatment in Homa Bay was significantly associated with high-grade fever ≥39^{0}C (aOR=3.1; 95% CI: 1.6-6.0), a history of fever lasting a week or more (aOR=5.0; 95%CI: 1.9-12.8), and presence of at least one IMCI danger sign (aOR=8.5; 95%CI: 4.8-14.9). Notably, there was no significant association of malaria over-treatment with HIV-infection. Results of multivariate analysis of malaria over-treatment are also displayed in Figure 4 by site.

Table 6: Univariate and multivariate analysis of correaltes of over-treatment in Homa Bay

	Over-treated				Crude	Adjusted[§]
Variable	**No (n=157)**		**Yes(n=210)**		**cOR(95% CI)**	**aOR(95% CI)**
Potential correlates	**n**	**(%)**	**n**	**(%)**		
Malnutrition						
MUAC<12.5	4	(2.5)	10	(4.8)	1.9 (0.6-6.2)	1.7 (0.4-8.1)
Wasting	17	(11.4)	26	(13.6)	1.2 (0.6-2.4)	0.7 (0.3-1.8)
Stunted	19	(12.5)	33	(17.1)	1.4 (0.8-2.7)	1.2 (0.5-2.9)
Underweight	14	(9.2)	27	(14.0)	1.6 (0.8-3.2)	0.9 (0.3-2.9)
Mother HIV-infected	30	(19.2)	45	(22.0)	1.2 (0.7-2.0)	1.2 (0.6-2.5)
Child HIV-infected	6	(3.9)	15	(7.1)	1.9 (0.7-5.0)	1.4 (0.4-5.3)
Any IMCI danger sign?	41	(26.1)	141	(67.1)	5.8 (3.7-9.2)	8.5 (4.8-14.9)
High grade fever (>39°C)	30	(19.1)	69	(32.9)	2.1 (1.3-3.4)	3.1 (1.6-6.0)
Fever for 7 or more days	10	(6.4)	35	(16.7)	2.9 (1.4-6.1)	4.9 (1.9 -12.9)
Sought care elsewhere	48	(30.6)	37	(17.6)	0.5 (0.3-0.8)	0.3 (0.2-0.6)
Taken Antimalarial last 7d	26	(16.6)	50	(23.8)	1.6 (0.9-2.7)	2.0 (1.0-4.0)
Taken Antibiotics last 7d	15	(9.6)	9	(4.3)	0.4 (0.2-.9)	0.17(0.1-0.6)

[§]Adjusted for child's age, sex, and caregivers' education and household monthly income.

Figure 4: Predictors of malaria over-treatment by site



Antibiotic treatment: We evaluated whether over-treatment of febrile children with antimalarials was as a result of these children being the sickest and being empirically treated with both antimalarials and antibiotics. Significantly more children with both a negative blood smear and RDT test result were treated with both antimalarials and antibiotics in Homa Bay than in Kisii (45.8% vs. 6.3%; p<0.001). In addition, in Homa Bay, children more likely to be treated with both antimalarials and antibiotics included those who presented with a history of fever lasting a week or more (OR= 2.4; 95%CI: 1.1-5.2) or manifested an IMCI danger sign(s) (OR=4.6; 95% CI: 2.8-7.2). In Kisii, although children presenting with high-grade fever of ≥39^{0}C but without malaria were more likely to receive both antimalarials and antibiotics (OR=2.3; 95%CI: 1.2-4.2), the presence of IMCI danger signs was not predictive of dual treatment (OR=1.2; 95% CI: 0.6-2.7).

Discussion

Febrile children presenting to rural hospitals in Kenya continue to be treated empirically for malaria despite guidelines recommending laboratory confirmation of parasitemia prior to treatment with antimalarials. Baseline malaria endemicity appears to be a major driver of over-treatment, particularly among children presenting with signs of more severe illness.

Among febrile children with laboratory-confirmed malaria, clinicians at both sites often accurately diagnosed malaria (73.0% in Kisii and 95.3% in Homa Bay). In an area of low malaria endemicity (Kisii) clinicians misdiagnosed relatively few (6.8%) children truly without malaria as having malaria, while clinicians in an area of high malaria endemicity (Homa Bay) incorrectly classified most of these children as having malaria (66.3%). As a result, in Homa Bay, more than half (57.2%) of febrile children without malaria were prescribed antimalarials compared to 7.0% in Kisii. Only a temperature of $>39^0$C was a strong predictor of inappropriate treatment with antimalarials in Kisii, while in Homa Bay, signs of more severe illness such as fever lasting a week or more, high-grade fever($>39^0$C), and presence of IMCI danger signs were predictors of inappropriate treatment for malaria. At both sites, children with markers of severe illness were likely to be treated empirically with both antimalarials and antibiotics.

The results of this study are consistent with studies in Nigeria[2], Uganda[30], and Zambia[73]. In an area of high malaria prevalence (Entomological Inoculation Rate >200) of Nigeria[2], over 80% of the smear negative children ≤12 years were treated with antimalarials, and children ≤5 years were more likely to be over-treated for malaria (OR=6.8; 95% CI: 5.5-8.3)[2]. In areas of Zambia with varied malaria endemicity, despite the availability of routine malaria diagnostic tests, antimalarials were prescribed for 58.4% of the febrile patients with negative smear results and for 35.5% of those with negative RDT results[73]. Finally, in Uganda[30], more than half of

febrile pediatric patients with negative blood smears received antimalarials, and children under the age of five were more likely than older children to be over-treated (RR=1.4, 95%CI: 1.2-1.5).

Our study adds to literature that clinicians continue to empirically treat fever with antimalarials, despite negative laboratory tests. Other studies have also explored factors underlying malaria over-treatment. In Uganda, clinicians prescribed antimalarials for children despite negative test results due to perceived high risk of mortality if therapy was delayed[30]. Qualitative studies exploring determinants of inappropriate antimalarial prescribing have found that clinicians' decision to over-treat is influenced by providers' clinical knowledge and beliefs of clinical expectations, high expectations by the patients to be informed as to the cause of their illness, limited understanding of malaria diagnostic testing and lack of diagnostics for identifying alternative causes of fever[32, 33]. In addition, patients often request treatment for malaria despite a negative malaria test because they strongly believe they need to be treated for malaria. While the impact of caregiver requests for antimalarials on treatment decisions was not evaluated in our study, this is an important driver of clinical treatment decisions[32].

We found that children exhibiting signs or symptoms consistent with severe disease (higher temperature, longer duration of fever, IMCI danger signs) were more likely to be treated for both malaria and for presumptive bloodstream infection. Although this study found relatively low rates of bacterial bloodstream infections (<5%), most children (>80% in both sites) were presumptively prescribed antibiotics. These findings suggest that in the absence of point-of-care diagnostics for other causes of fever, clinicians are reluctant to miss the opportunity to avert potential morbidity and mortality and will presumptively treat children, even when guidelines suggest otherwise. Thus, over-treatment of malaria may continue despite declines in malaria incidence and as the proportion of fever due to other causes increase[8]. However, our observation of lower rates of over-treatment in a region with lower malarial prevalence suggests

that eventually over-treatment rates will decline as cases of true malaria also become less frequent.

While this study had several strengths, notably the inclusion of data from two sites in Kenya with differing underlying malaria endemicity, there were also some weaknesses. We only evaluated malaria over-treatment in relation to patients' demographic and clinical symptoms. Other factors such as clinicians' level of training, number of clinical staff and patient load, patients' expectations, diagnostic laboratory capacity and supervision and public health promotional activities likely affect clinicians' malaria diagnosis and treatment decisions. We did not collect outcome or anti-malarial resistance data; we speculate that over-treatment leads to increased individual and community wide resistance. While clinicians followed protocols for management of fever, it is possible that the clinical decisions were specific to these clinicians. It is also possible that other sites would have different treatment practices. Overall, we believe that our results are generalizable and can inform better training and guidelines for clinicians to reduce the risk of antimalarial resistance and missed treatment opportunities.

Conclusions

Malaria was treated, despite negative laboratory testing, more frequently in Homa Bay than in Kisii, likely due to underlying higher regional malaria prevalence. Despite the Kenyan Ministry of Health's revised malaria treatment guidelines and the availability of diagnostic testing, malaria prevalence appears to influence clinical management of children in Kenya and may result in missed opportunities to accurately diagnose alternative causes of fever. This lack of adherence to existing guidelines in the face of recent dramatic declines in malaria suggests the need for the strengthened training on fever case management, supervision, monitoring and evaluation of malaria treatment practices by clinicians in order to ensure adherence to guidelines**,** particularly in areas of high malaria endemicity. The revised guidelines and the wide deployment of rapid

diagnostic tests for malaria to health facilities may not achieve their full benefits if not accompanied by changes in practice by clinicians. Because of concerns of emergence of resistance to artemesinin-based combination therapies (ACTs), unnecessary side effects and the need for proper management of non-malarial febrile illnesses, it is important to prioritize malarial treatment for patients who test positive for malaria.

Chapter 5: Bacteremia Among Febrile Children in Areas of Differing Malaria Transmission in Rural Kenya

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Abstract

Background: With malaria declining, other causes of fever may account for a substantial portion of severe childhood illness in sub-Saharan Africa. We determined the prevalence and correlates of bacteremia among febrile children in Western Kenya.

Methods: Children aged 6 months to 15 years presenting with fever to Kisii Hospital (a low malaria endemic area with entomological infection rates; EIR <1.5) and Homa Bay Hospital (a high malaria endemic area; EIR ≥300) were enrolled and screened for malaria, HIV and bacteremia. Predictors of bacteremia were evaluated using multivariate logistic regression.

Findings: Among 1476 children enrolled, 48 (3.3%) had culture-confirmed bacteremia (3.1% in Kisii and 3.4% in Homa Bay). *Salmonella spp.* (19 *typhi* and 20 nontyphoidal salmonella [NTS]) accounted for 81% of isolates. The distribution of *Salmonella spp* did not differ between sites. Bacteremia prevalence in children with and without malaria was 1.9% and 3.8%, respectively (p=0.05); co-infection of bacteremia and malaria was uncommon (<0.5%). Bacteremia was associated with incomplete vaccination (adjusted Odds Ratio [aOR]=2.1; 95% CI: 1.1-4.1), the use of antimalarials before hospitalization (aOR=2.7; 95%CI: 1.4-4.1), having sought care elsewhere (aOR=2.2; 95% CI: 1.2-4.0) and lower education of caregiver (aOR=2.5; 95% CI: 1.1-4.8). NTS bacteremia was associated with HIV-infection (aOR=6.2; 95% CI: 1.1-35.1) and anemia (aOR=5.2; 95% CI: 1.4-18.9).

Interpretation: Bacteremia was relatively uncommon in both sites. However, children with HIV, with anemia, those who are incompletely vaccinated or those with persistent fever despite malaria treatment, may have higher risk and may benefit from targeted bacterial culture and/or empiric antibiotic therapy.

Key words: fever, malaria, bacteremia, Africa

Background

Febrile illness is a leading cause of morbidity and mortality among children of sub-Saharan Africa (SSA)[1]. Historically, a large proportion of febrile illness in SSA was due to malaria. However, substantial reductions in malaria incidence have been observed in many regions over the past decade[4, 20]. In addition, as malaria rapid diagnostic tests are increasingly available in many settings, malaria is more easily excluded as a cause of fever.[8] Community-acquired bacteremia has emerged as a major cause of non-malarial febrile illness among African children, with mortality exceeding 40%, most of which occurs in the first 48 hours of hospitalization[9, 10, 15, 41]. The majority of health facilities in rural areas of SSA have limited laboratory capacity for alternative diagnoses of febrile illness, including bacteremia[74].

The proportion of febrile illness due to bacteremia may have changed in recent years following declines in malaria prevalence. Since malaria has been associated with risk of bacteremia, rates of bacteremia may be expected to decline concurrently with reductions in incident malaria.[4, 6, 8, 70, 75] In addition, pathogen distribution and antibiotic susceptibility profiles may also have changed following widespread introduction of *Haemophilus influenzae type b* and pneumococcal vaccines, and the growing population of HIV-infected individuals on prophylactic cotrimoxazole[18].

Studies that that have attempted to assess bacteremia as a cause of acute pediatric febrile illness have focused primarily on children admitted to urban referral hospitals or established research facilities[15, 74, 76]. Given regional and rural-urban differences in rates and etiologies of bacteremia[8, 18], and differences in prevalence of potential risk factors of bacteremia, including HIV, malnutrition, and malaria, existing studies may not be generalizable to children in other areas. We determined the prevalence, causative organisms, and correlates of bacteremia in

consecutive febrile children presenting to two regional rural hospitals in Western Kenya to inform optimal management of pediatric febrile illness in this setting.

Materials and Methods

Study sites and settings

The study was conducted at Kisii Provincial and Homa Bay District Hospitals in rural Western Kenya. These hospitals serve regions that are geographically and demographically distinct, with significant differences in the prevalence of HIV and malnutrition.[63, 64, 77] Malaria transmission in Kisii is seasonal and low (hypoendemic). The annual Entomological Inoculation Rate (EIR) in Kisii is 0.4-1.5 infectious mosquito bites per person per year[57, 58]. Homa Bay experiences intense perennial malaria transmission (holoendemic for malaria)[60], with an EIR as high as 300 infectious mosquito bites per person per year[78]. The prevalence of HIV among antenatal care clients in Homa Bay is 28% and the highest in Kenya, compared to 4.6% in Kisii.[59, 79].

Study design, population and eligibility

This was a cross-sectional surveillance study of pediatric febrile illnesses. Over a two-year period (April 2012 to March 2014), children ages 6 months to 15 years presenting with an axillary temperature of ≥37.5^{0}C to outpatient clinics at each facility were approached consecutively for enrolment. Children were eligible to participate in the study if they were able to provide a blood sample, their parents or guardians were willing to provide written informed consent for them to participate in the study and agreed to attend directed HIV-1 counseling and testing (DCT). Assents were also obtained from children ≥13 years of age. Children were excluded if they were unaccompanied by biological parents or legal guardians to the hospital, had an obvious non-infectious cause for admission, such as trauma, surgery, or known malignancy; and/or if the parents/guardians were unwilling to consent to HIV counseling on

behalf of the child. The study was approved by the Research Ethics Committee's at the University of Washington and the Kenya Medical Research Institute.

Clinical data collection

Study staff administered standardized data collection instruments to obtain socio-demographic information, presenting clinical signs and symptoms, medical history and physical examination findings. All children were assessed using Integrated Management of Childhood Illness (IMCI) guidelines and those presenting with one or more of the IMCI clinical danger signs (unable to drink or breastfeed, convulsions, continuous vomiting, and/or lethargy, unconsciousness) were classified as having severe febrile illness[80]. Blood was also collected for complete blood count, malaria, HIV, and blood culture testing. Children with suspected bacteremia received empiric treatment with antibiotics immediately following blood collection according to Kenya Ministry of Health guidelines[66]. Community-acquired bacteremia (CAB) was defined as a positive blood culture due to a known pathogenic (non-contaminant) bacteria obtained within the first 48 hours of hospital admission from a child with no history of previous hospitalization.

Laboratory data collection

Blood Cultures and identification of isolates: Up to 3 mL of venous blood specimens were collected using standard BACTEC Peds blood culture bottles and shipped to the US-Army Microbiology laboratory in Kericho, Kenya, within 24 hours of collection. Cultures were incubated in a BD BACTEC™ 9050 automated blood culture system. Bottles were considered negative if they did not signal within 5 days of incubation at 35^0C, and were discarded. Blood cultures flagged as positive were gram stained and sub-cultured onto appropriate media; blood agar and any nutrient agar for gram positive bacteria, and chocolate agar, MacConkey, sorbital MacConkey, Hectoin agar for gram negative bacteria. Sub-cultured isolates were immediately incubated for 24-48 hours to obtain pure bacteria colonies that were subjected to bacterial

identification and antibiotic susceptibility testing using a Microscan Walkaway40® plus system. Positive blood cultures were classified as probable pathogens, possible pathogens or likely contaminants[81] by infectious disease specialists at the University of Washington. Patients with positive blood cultures who were not admitted to hospital wards were immediately recalled to the hospital for appropriate antimicrobial therapy if deemed necessary by the study clinical staff.

HIV Testing: Per Kenya Ministry of Health guidelines, all enrolled children over 18 months of age were tested for HIV using Abbott Determine™ rapid test kit and confirmed using Uni-Gold™ if positive. Children <18 months were tested using RNA PCR. A child was classified as HIV-exposed if he or she was accompanied by a biological mother who agreed to be tested and tested HIV-positive or who reported being HIV-positive on prior testing.

Malaria Diagnosis: Blood was evaluated for malaria using thin and thick smears and Paracheck Pf® rapid diagnostic testing (Orchid Biomedical Services, India). Malaria was defined as having a positive smear or RDT.

Statistical Analysis

Sociodemographic, clinical and laboratory characteristics were summarized and compared between study sites. Continuous variables were summarized using mean and standard deviation and compared between children with and without bacteremia using the two-sample t-test. Categorical variables were summarized using counts and proportions and compared between children with bacteremia and those without bacteremia using Pearson's chi-square tests or Fisher's exact tests.

Height for age z-score (HAZ) weight for height z-scores (WHZ) and weight-for-age z-scores (WAZ) were calculated using the 2006 and 2007 World Health Organization (WHO) reference

populations for children aged 6 months to <5 and ≥5 respectively[82, 83]. Stunting, wasting, and underweight were defined as HAZ <-2, WHZ <-2 and WAZ<-2, respectively. A child was considered malnourished if he or she was classified as either wasted, underweight or stunted or had a mid-upper arm circumference (MUAC) <12.5cm.

We determined the prevalence of bacteremia for all children after excluding potential contaminants. Because we were also interested in evaluating bacteremia as a cause of non-malarial fever, we evaluated several potential risk factors (demographic, host and environmental) for all children, and in a subgroup of febrile children with negative malaria tests results using logistic regression models with bacteremia as dependent variable. First, we used bivariate logistic models to evaluate the unadjusted associations of bacteremia and several potential predictors that we had selected *a priori* based on the current literature. These included HIV, malaria, anemia, malnutrition, pre-treatment with antimalarials or antibiotics, high or prolonged fever, presence of IMCI danger signs, vaccination statuses, exposure to animals, overcrowded living, consumption of unsafe water, caregivers' education. To identify independent predictors of bacteremia, we adjusted for the following a priori selected confounders in the multivariate logistic model; child's age, sex, and study site. Unadjusted and adjusted odds ratios with 95% confidence intervals were computed using the logistic regression models. Analyses were conducted using Stata statistical software (version 13.1, Stata Corp., College Station, TX).

Results

Demographic, medical history, and clinical characteristics

Between April 2012 and March 2014, 1605 children presented with fever to the study hospitals, of which 1478 were eligible for inclusion, and 1476 were enrolled into the study (Figure 5). Sociodemographic, medical and clinical characteristics of the enrolled children are presented by

enrollment site in Table 6. Compared to children from Kisii, children from Homa Bay were significantly younger (mean age: 33.9 vs. 36.8 months; p=0.014) and were more likely to be female (p=0.05), to have primary caregivers with lower education levels (p<0.001), to live in lower-income households (<500 Kenya shillings/month) and more crowded households (p<0.001), to consume water from unimproved sources (p<0.001) and were more likely to have travelled for ≥30 minutes to hospital (p<0.001). Children from Homa Bay were less likely than those from Kisii to have received all the age appropriate vaccinations per the Kenya National Expanded Program of Immunization (KEPI) (34.6% vs. 12.0%; p<0.001).

Children in Homa Bay were more severely ill at based on the presence of ≥1 IMCI danger signs (51.5% vs. 16.9%; p<0.001) and were more likely to be malaria-infected (49.2% vs. 8.6%; p<0.001), HIV-infected (4.5% vs. 1.2% p<0.001), or HIV-exposed (19.3% vs. 3.4%; p<0.001). In addition, children from Homa Bay were less likely to have previously sought healthcare elsewhere for their current illness (24.3% vs. 34.7%; p=0.001).

Prevalence of bacteremia, and causative organisms

Overall, 94 (6.4%) of 1476 children with culture data available had positive blood cultures (Table 7). Of these, 46 isolates were likely contaminants (overall contamination rate of 3.1%; Kisii 4.8% (35/734) and Homa Bay 1.5% (11/742); p<0.001). Considering likely contaminants as negative blood cultures; the prevalence of bacteremia was 3.3% (48/1476) overall; Kisii 3.1% (24/734) in and Homa Bay 3.4%(25/742). Salmonella species (spp). (21 non-typhoidal salmonella (NTS) and 20 *salmonella typhi*) were the most commonly isolated organisms, accounting for 81.3% (40/48) of all isolates (Table 7). The second most species identified was streptococcus spp. (2 S. *pneumoniae* and 1 S. *pyogenes*). There were no differences in the distribution of pathogens between the study sites.

Over two-thirds (71.5%) of enrolled children had fever due to causes other than malaria or

bacteremia. Of enrolled children, 28.5% had malaria only, 2.7% had bacteremia only, and 0.5% children had both confirmed malaria and bacteremia. Bacteremia was more common among febrile children without malaria than in those with malaria (3.8% vs. 1.9%; p=0.05). Although antibiotics were frequently given to children with confirmed malaria (55.7%), antibiotics were presumptively prescribed to more frequently (93.1%) in children without malaria; p<0.001.

Figure 5: Recruitment into the bacteremia study

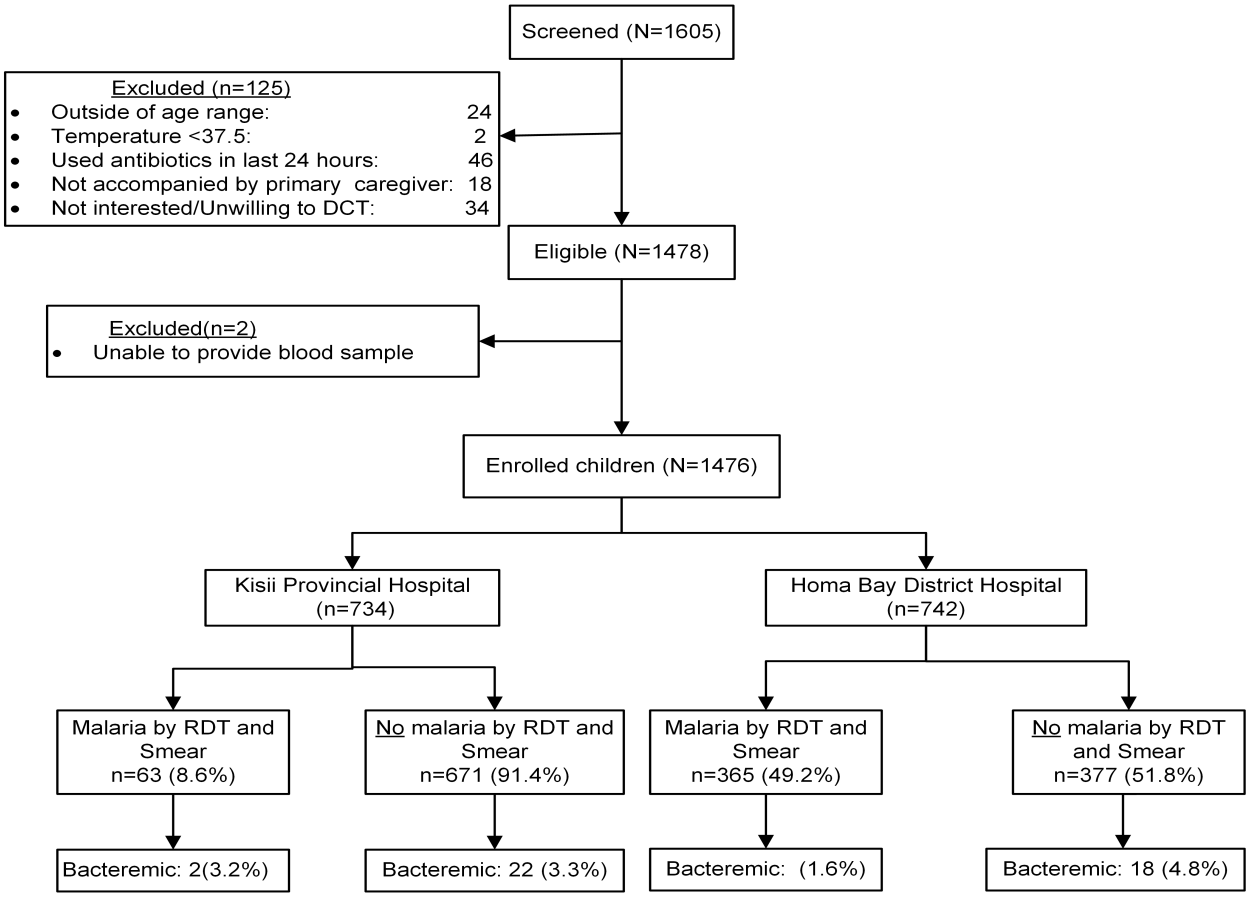


Table 7: Demographic and clinical characteristics of study children and caregivers

Variable	Study Site				p
	Kisii (n=732)		Homa Bay (n=742)		
	n	%	n	%	
Female sex	330	(45.0)	371	(50.0)	0.053
Age in months (Mean±SD)(median)	36.8±24.6	(35.0)	33.9±18.6	(33.0)	0.014
Child has visible severe wasting?	10	(1.4)	21	(2.8)	0.04
Consumes water from unimproved sources	28	(3.8)	162	(21.8)	<0.001
Exposed/in close contact with farm animals	550	(74.9)	588	(79.2)	0.049
Overcrowding living (>2/room)	158	(21.5)	382	(51.5)	<0.001
Not received all age-appropriate vaccines	88	(12.0)	257	(34.6)	<0.001
Travel time to the hospital ≥30 minutes	347	(47.3)	445	(60.0)	<0.001
Caregiver's demographic characteristics					
Primary caregiver					0.152
Biological mother	672	(91.9)	700	(94.5)	
Biological father	34	(4.7)	23	(3.1)	
Legal guardian	25	(3.4)	18	(2.4)	
Marital status					0.151
Single	41	(5.6)	59	(8.0)	
Married	657	(89.5)	653	(88.0)	
Widowed/Divorced	36	(4.9)	30	(4.0)	
Education level					<0.001
≥ Secondary	408	(55.6)	231	(31.1)	
≤Primary	326	(44.4)	511	(68.9)	
Household income < Ksh. 5000(USD <60)	197	(26.9)	402	(54.2)	<0.001
Medical history/clinical characteristics					
Malnutrition indicators					
MUAC<12.5	16	(2.2)	26	(3.5)	<0.049
Wasting(WHZ<-2)	122	(19.1)	84	(12.1)	<0.001
Stunted(HAZ<-2)	91	(13.8)	106	(15.1)	0.478
Underweight(WAZ<-2)	55	(8.3)	79	(11.3)	0.068
Mother infected with HIV	24	(3.4)	140	(19.3)	<0.001
Child infected with HIV	9	(1.2)	31	(4.2)	<0.001
High grade fever (>39°C)					<0.001
Fever for >=7 days	273	(37.2)	90	(12.1)	<0.001
Used antimalarials in past 7days	92	(12.5)	112	(15.1)	0.154
Used antibiotics in past 7days	60	(8.2)	40	(5.4)	0.033
WHO/MCI-defined pneumonia	9	(1.2)	242	(32.6)	<0.001
Any IMCI danger sign present	124	(16.9)	382	(51.5)	<0.001
Unable to drink/breastfeed	14	(1.9)	186	(25.1)	
Vomits everything	68	(9.3)	232	(31.3)	
Convulsions	34	(4.6)	53	(7.5)	
Lethargic/unconscious	49	(6.7)	71	(9.6)	
Sought care elsewhere	255	(34.7)	180	(24.3)	<0.001
Where sought care prior to hospital visit:					
Traditional healer	9	(3.5)	3	(1.7)	
Chemist	112	(43.9)	88	(48.9)	
Health Centre /Dispensary/Clinic	131	(51.4)	84	(46.6)	
Other hospital	3	(1.2)	5	(2.8)	
Has chronic Disease (sickle-cell/heart disease)	3	(0.4)	19	(2.6)	0.001
Malaria by RDT or Smear	63	(8.6)	365	(49.2)	<0.001

Table 8: Prevalence of bacteremia and relative importance of causative agents

		Kisii	Homa Bay	All
		n=734	n=742	n=1476
Any Pathogenic Bacteremia		**24(3.2%)**	**25(3.5%)**	**48(3.3%)**
Pathogen				
Salmonella		**16**	**23**	**40**
	NTS	8	11	19
	Typhi	8	13	21
Staphylococcus		**2**	0	**2**
	Aureus	2	0	2
Streptococcus		**2**	**0**	**2**
	Pneumoniae	2	0	2
	Pyogenes	0	1	1
Escherichia coli		**2**	0	**2**
Hemophyllus	Unspecified	1	0	0
Potential Contaminants		**35(4.8%)**	**11(1.5%)**	**46 (3.1%)**
Micrococcus		7	5	12
Staphylococcus	Epidermidids	3	0	3
	Hominis	3	1	4
	Auricularis	2	0	2
	Capitis	1	0	1
	Sciuri	1	1	2
	Intermedius	0	1	1
Streptococcus	Anginosus	1	0	1
	Bovis	4	1	5
	Parasanguis	1	0	1
	Mutans	0	1	1
Gram positive rods	Unspecified	9	0	9
Gram negative rods		1	0	1
Gram variable rods		1	0	1
Pleomorphic bacilii		1	0	1
Acinetobacter Lwoffii		0	1	1

Correlates of bacteremia due to any pathogen

Results of bivariate and multivariate analyses of correlates of bacteremia are presented in Table 8. In bivariate analysis, bacteremia was positively associated with a history of pre-treatment with antimalarials in the preceding week (crude odds ratio [cOR]=3.0; 95%CI: 1.6-5.6), having sought health care elsewhere (OR=2.3; 95% CI: 1.3-4.0) and low education (≤ primary level) of caregiver (cOR=2.4; 95%CI: 1.2-4.6). Bacteremia was inversely associated with malaria parasitemia (cOR=0.5; 95% CI: 0.2-0.9). In multivariate logistic model, bacteremia was

significantly associated with non-receipt of all required age-appropriate vaccinations (adjusted odds ratio [aOR]= 2.1; 95% CI: 1.1-4.1), pre-treatment with antimalarials prior to enrollment (aOR=2.5; 95%CI: 1.4-4.8), having sought healthcare elsewhere (aOR=2.2; 95% CI: 1.2-4.0), low education of caregiver (≤primary level) (aOR=2.3; 95% CI: 1.1-4.8) and inversely associated with malaria parasitemia (aOR=0.37; 95% CI: 0.2-0.8). Odds of bacteremia were lower for children with malaria infection (aOR=0.4; 95% CI: 0.2-0.8) or with a history of prolonged fever lasting at least a week (aOR=0.4; 95% CI: 0.2-0.9). No association was seen between bacteremia and HIV, nutritional status, consumption of unsafe water, contact with farm animals, overcrowding, or more severe febrile illness.

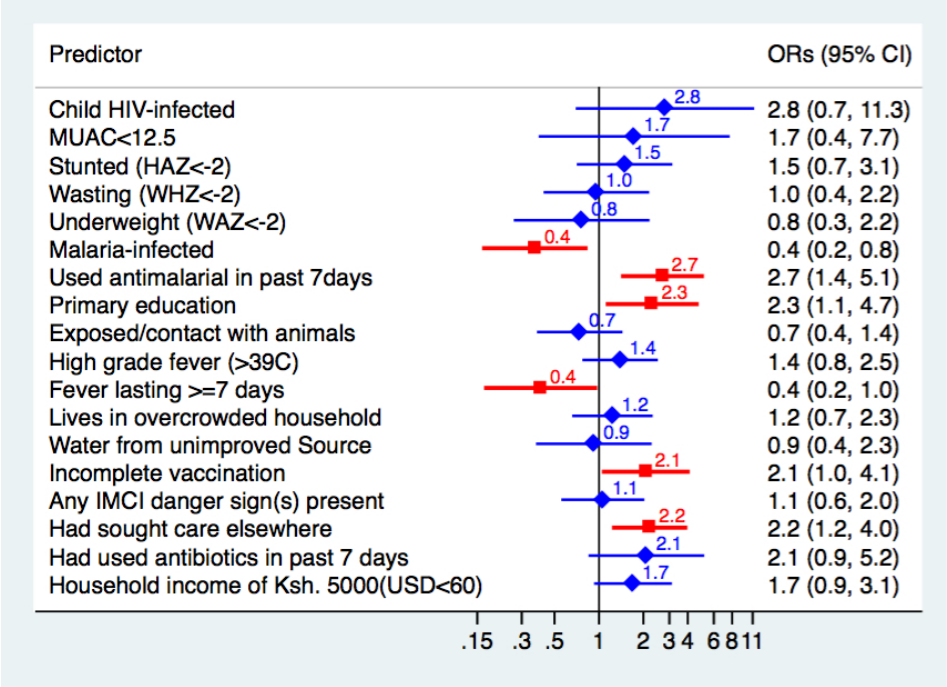
When the analysis was restricted to children without malaria, treatment with antimalarials in the week prior to hospitalization (aOR=2.9; 95% CI: 1.4-5.8), previous history of care seeking (aOR=3.2; 95% CI: 1.7-6.1) and low education (≤ primary level) of caregiver (aOR=2.7; 95% CI: 1.2-5.6) significantly predicted bacteremia in both univariate and multivariate analyses. Results of multivariate analysis of the predictors of bacteremia due to any pathogen are also displayed in Figure 6 below.

Table 9: Univariate and multivariate analyses of correlates of bacteremia

Variable	Bacteremia present			
	No (n=1428)	Yes (n=48)	OR(95%CI)	aOR(95%CI)[§]
Child HIV-infected				
No	1375(97.4)	43(93.5)	1	1
Yes	37(2.6)	3(6.5)	2.6 (0.8-8.7)	2.8 (0.7-11.3)
Malnutrition indicators				
MUAC<12.5	40(2.8)	2(4.2)	1.5 (0.4-6.4)	1.9 (0.4-8.6)
Stunted (HAZ<-2)	188(14.3)	9(22.0)	1.7 (0.8-3.6)	1.7 (0.8-3.6)
Wasting (WHZ<-2)	201(15.6)	5(12.2)	0.8 (0.3-1.9)	0.8 (0.3-2.2)
Underweight (WAZ<-2)	130(9.9)	4(9.8)	1.0 (0.3-2.8)	1.0 (0.3-2.8)
Contact with animals				
No	325(22.8)	13(27.1)	1	1
Yes	1103(77.2)	35(72.9)	0.8 (0.4-1.5)	0.7 (0.4-1.4)
Sources of drinking water				
Improved Source	184(12.9)	6(12.5)	1	1
Unimproved Source	1244(87.1)	42(87.5)	1.0 (0.4-2.3)	0.9 (0.4-2.3)
Overcrowded living (>2/room)				
No overcrowded	908(63.6)	28(58.3)	1	1
Overcrowded	520(36.4)	20(41.7)	1.2 (0.7-2.2)	1.2 (0.7-2.3)
Got all age-appropriate vaccines				
Yes	1098(76.9)	33(68.8)	1	1
No	330(23.1)	15(31.2)	1.5 (0.8-2.8)	**2.1 (1.1-4.1**)
High grade fever (>39C)				
Temperature <39C	932(65.3)	29(60.4)	1	1
Temperature >39C	496(34.7)	19(39.6)	1.2 (0.7-2.2)	1.2 (0.8-2.5)
Fever for 7 or more days				
Fever for <7days	1071(75.0)	42(87.5)	1	1
Fever for >=7 days	357(25.0)	6(12.5)	0.4 (0.2-1.0)	**0.4 (0.2-0.9)**
Used antimalarial in past 7days				
No	1238(86.7)	34(70.8)	1	1
Yes	190(13.3)	14(29.2)	**2.7 (1.4-5.1)**	**2.5 (1.3-4.8)**
Used antibiotics in past 7 days				
No	1331(93.4)	45(93.8)	1	1
Yes	97(6.8)	3(6.3)	0.9 (0.3-3.0)	0.9 (0.3-3.1)
Any IMCI danger sign				
Absent	939(65.8)	31(64.6)	1	1
Present	489(34.2)	17(35.4)	1.1 (0.6-1.9)	1.0 (0.6-2.0)
Sought care elsewhere				
No	1015(71.1)	26(54.2)	1	1
Yes	413(28.9)	22(45.8)	**2.1 (1.2-3.7)**	**2.2 (1.2-4.0)**
Malaria-infected (any test)				
Negative	1008(70.6)	40(83.3)		1
Positive (any test)	420(29.4)	8(16.7)	**0.5 (0.2-0.9)**	**0.4 (0.2-0.8)**
Severe or moderate anemia				
No (haemoglobin ≥8 g/dL)	1382 (96.8)	45(93.8)	1	**1**
Yes (haemoglobin <8 g/dL)	46 (3.22)	3(6.3)	2.0 (0.6-6.7)	**2.4 (0.7-8.1)**
Education				
≥Secondary	627(43.9)	12(25.0)	1	1
Primary	801(56.1)	36(75.0)	**2.3 (1.2-4.6)**	**2.5 (1.2-4.8)**
Income				
≥Ksh. 5000(USD<60)	851(59.7)	24(50.0)	1	1
< Ksh. 5000(USD≥60)	575(40.3)	24(50.0)	1.5 (0.8-2.6)	1.7 (0.9-3.1)

[§]Adjusted for age, sex, site, mother's HIV status

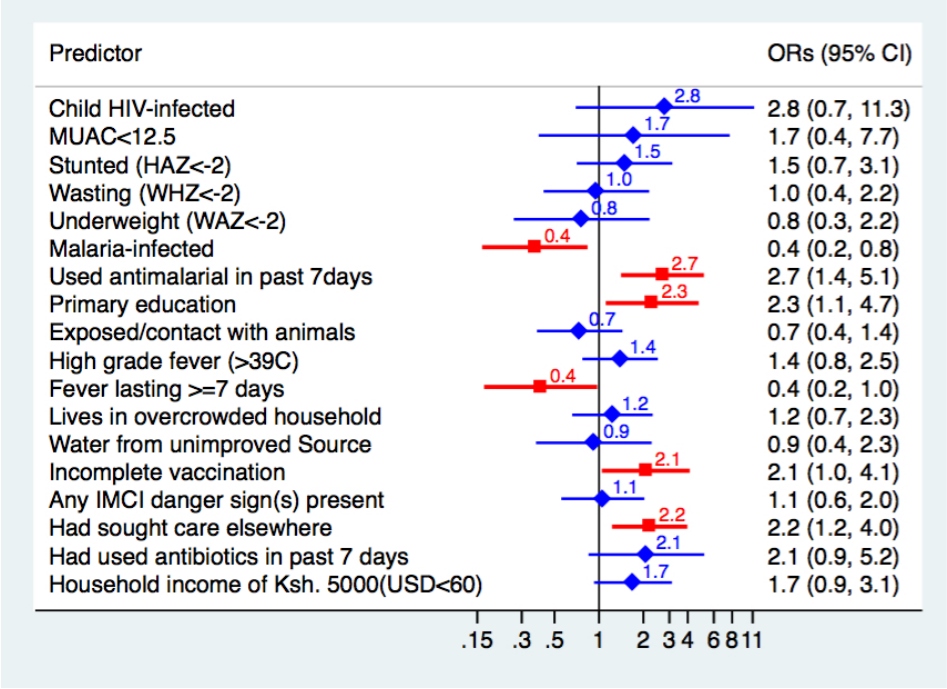
Figure 6: Predictors of bacteremia infection among febrile children



Correlates of bacteremia due to NTS and salmonella typhi

Because bacteremia due to NTS and salmonella typhi have different risk factors and pathogenesis, we analyzed evaluated NTS and salmonella typhi as separate outcomes[51]. When compared to other isolated pathogens, NTS was strongly associated with malaria parasitemia (aOR=7.8; 95% CI: 1.4-42.6). Although the limited prevalence (1.4%; 20/1476) of NTS bacteremia precluded an exhaustive analysis of risk factors for NTS, in univariate analysis, NTS bacteremia was associated with non-receipt of vaccines (cOR=3.0; 95%CI: 1.2-7.5), hemoglobin levels <8 g/dL (cOR=5.8; 95% CI: 1.6-20.4), and low household monthly income (cOR=3.2; 95%CI: 1.2-8.5) and with a trend to be associated with HIV (cOR=4.1; 95%CI: 0.9-18.3). In multivariate analysis adjusting for age and sex, site of enrolment and mother's HIV status, NTS was also associated with HIV-infection (aOR=6.8; 95% CI: 1.2-38.8), and anemia (aOR=5.2; 95% CI: 1.4-18.9); but was not with prior recent malaria infection (Figure 7).

Figure 7: Predictors of non-typhoidal salmonella (NTS) bacteremia



In univariate analysis, bacteremia due to salmonella typhi was strongly associated with recent treatment for malaria (OR=4.7; 95%CI: 1.9-11.8) and contact with animals (cows) (OR=2.7; 95% CI: 1.1-6.8). In multivariate analysis, bacteremia due to salmonella typhi was strongly associated with recent treatment for malaria (OR=3.3; 95%CI: 1.2-8.8) and contact with animals (cows) (OR=2.6; 95% CI: 1.0-6.7).

Discussion

As improvements in the detection and management of malaria continue to reduce malaria incidence, there is increasing need to diagnose and treat other causes of fever. We determined the prevalence and correlates of bacteremia in children presenting to hospitals in settings of differing malaria transmission in rural Western Kenya. In this cohort, bacteremia was relatively uncommon; only 3.3% of all children had a pathogen isolated from blood culture. The proportion of children with malaria and bacteremia co-infection was also low in this cohort (<0.5%). *Salmonella spp*. (including non-typhoidal salmonella (NTS) and *Salmonella typhi*) were predominant causes of bacteremia in this study.

The prevalence of bacteremia in this study was similar to that reported in a similar cohort of febrile Tanzanian children.[6] However, the prevalence is lower than has been reported in other African studies, which have noted rates of bacteremia as high as 15% in cohorts of febrile children[10, 74, 84, 85], and high rates of childhood bacteremia in areas of high malaria endemicity[51]. Studies conducted in malaria-endemic sites in Africa have demonstrated declining incidence over time of overall bacteremia, and NTS bacteremia following declining malaria incidence[75, 86]. From 2006 to 2010, declines in malaria incidence among Tanzanian children <15 years of age, from 504 to 106 per 100,000 children, was associated with reductions in NTS bacteremia incidence from 82 to 7 per 100,000 children[86]. In Tanzania, viral infections were found to be responsible for a larger proportion of cases of febrile illness in pediatric outpatients than bacteremia or malaria[7]. In Kenya, pediatric hospitalizations for bacteremia also have declined since 1999, paralleling declines in malaria[75]. Our results, taken together with previous studies, provide further evidence that bacteremia may be declining as a cause of fever in African children, concurrent with declines in malaria.

Bacteremia in this cohort was associated with incomplete vaccination, having previously sought healthcare, prior treatment with antimalarials and low education of the primary caregiver. These factors suggest that risk of bacteremia may be associated with poor engagement with the health care system. Incomplete vaccination may be a proxy for limited health care exposure and caregivers with less education may be more likely to delay health care seeking, choosing to first manage fever at home. The association of bacteremia with use of antimalarials in the week preceding hospitalization also likely reflects inappropriate home treatment of bacteremic children. Caregivers with lower levels of education were more likely than those with higher education to have administered antimalarials to their children prior to presentation at the hospital. These findings suggest a need for targeted educational programs to improve care-

seeking behavior for sick children, particularly among parents with low education.

Consistent with other studies, HIV-infected children in this study had nearly a 7-fold increased odds of NTS bacteremia[9, 87, 88] and NTS bacteremia was strongly associated with malaria parasitemia and anemia[89, 90]. We did not find associations of NTS bacteremia with nutritional status, consumption of unsafe water, incomplete vaccination, or more severe illness, findings that differ from previous reports[9, 91] The relatively few NTS isolates identified may have resulted inadequate statistical power to detect these associations.

More than two-thirds of the children in this study had fever attributable to causes other than malaria or bacteremia. In absence of clear guidelines for the management of febrile children who test negative for malaria, prescription of antibiotics and/or antimalarials to febrile children remains common[6]. These findings suggest that in the absence of signs of severe illness or important comorbidities, and in the presence of a negative test for malaria, many febrile outpatient children may not benefit from antibiotic therapy[59]. Despite the low prevalence of bacteremia, more than 80% of the children in this study were presumptively prescribed antibiotics. The apparent overtreatment of many children without severe bacterial infection is likely to contribute to the emergence of antimicrobial resistance.

This study had several strengths, notably the inclusion of two rural sites with varied prevalence of both HIV and malaria and the careful assessment of multiple risk factors for childhood bacteremia. Identification of causative organisms and antimicrobial susceptibility testing was performed using standard culture techniques in a high quality laboratory and the observed percentage of contaminants (2.9%) was lower than reported in previous studies (14.3%) in Kenya[9] or elsewhere in Africa[92]. However, there were also important limitations. The study was conducted at two sites in relatively close geographic proximity (70 Km apart) limiting some

generalizability of these findings. Although few caregivers reported recent use of antibiotics (~7%), self-reported history may not have reliably captured all antibiotic use. In addition, although bacterial cultures and malaria testing were performed, viral testing was not performed and the cause of fever was undiagnosed in over two-thirds of the children. Finally, the low rate of positive blood cultures limited the statistical power to explore associations with specific bacterial pathogens.

The temporal association between malaria and bacteremia has been well established[75, 86] and bacteremia in sub-Sahara Africa has recently been reported to be twice as common in an area of high malaria transmission intensity as in an area of low endemicity[51]. However, we found bacteremia prevalence to be similar between sites of low and high malaria transmission in Western Kenya. Although bacteremia in this study was generally uncommon, important subgroups of children including those presenting with severe illness, those with persistent fever despite antimalarials, those incompletely vaccinated, and those with HIV, anemia, and whose caregivers have low education may be at high risk. Interventions such as focused education to improve care-seeking, targeted microbiologic assessment and targeted empiric therapy may be beneficial in these children in light of the high risk of mortality due to bacteremia[9, 15]. Finally, we found that in an area of high malaria transmission (Homa Bay), bacteremia was thrice as common in children without malaria parasitemia as in children with malaria parasitemia (5.0% vs 1.6; $p<0.001$). This suggests that a high index of suspicion for bacteremia may be required among febrile children who test negative for malaria parasites, especially if they reside in an area of high malaria endemicity.

Chapter 6: Discussion and conclusions

The etiology and management of non-malarial febrile illness is increasingly becoming more important in malaria-endemic areas of sub-Sahara Africa where incidence of that disease continues to decrease. This study has provided information on the prevalence and predictors of malaria over-treatment, as well as bacteremia among children presenting with fever to regional hospitals in rural Western Kenya. Because a majority of children in sub-Saharan Africa live in rural areas such as the Kisii and Homa Bay, findings of from this study will have important practical public health implications in the management of children with febrile illnesses.

Malaria over-treatment

The New WHO malaria guidelines are intended to prevent over-treatment by ensuring that only febrile patients with malaria receive antimalarials, and that those without malaria are evaluated for other pathogens and treated appropriately or urgently referred for further management. We found that malaria prevalence appears to influence the clinical management of children in Kenya. Children residing in an area of intensive malaria transmission were frequently prescribed antimalarial drugs despite negative malaria test results; especially those children with signs of severe febrile illness often. This likely resulted in missed opportunities to accurately diagnose alternative causes of febrile illness fever, with severe consequences. Febrile children treated for malaria alone when they actually have no malaria infection is twice as high as in children who benefit from laboratory confirmed malaria.

In order for the new WHO malaria guidelines to achieve their full clinical and financial benefits, there is need for regular training of clinicians on fever management, supervision, monitoring and evaluation of malaria treatment practices by clinicians so as to ensure adherence to the guidelines, particularly in areas of high malaria endemicity. Additionally, because of concerns of

emergence of resistance to artemesinin-based combination therapies (ACTs), unnecessary side effects and the need for proper management of non-malarial febrile illnesses, it is important that antimalria drugs be restricted to only patients who test positive for malaria. The emergence of resistance ACTs is considered the greatest global threat to the future success of malaria control.

Community-acquired bacteremia

Community-acquired bacteremia has emerged as a one of the major causes of non-malarial febrile illness among African children with increased mortality. Our results provide further evidence to recent findings from multiple sites in Africa[7, 93] that bacteremia may becoming uncommon as a cause of febrile illness in children. We found that over two-thirds of the children had fever due to causes other than malaria or bacteremia. Despite the strong association of malaria endemicity and bacteremia, particularly non-typhoidal salmonella (NTS)[51], the prevalence of bacteremia due to any pathogen, or NTS was similar between sites of low and high malaria transmission in Western Kenya. However, certain subgroups of children may be at increased infection with bacteremia. This includes those children with severe febrile illness, those with persistent fever despite pre-hospital antimalarials use or having sought care elsewhere, those incompletely vaccinated, and those with HIV, anemia, and whose caregivers have low education may be at high risk. The characteristics of children more likely to present with bacteremia suggest delayed access to, or poor engagement with, the health care system.

Given that most health facilities in SSA have limited laboratory capacity to diagnose alternative causes of fever, these findings suggest that children with HIV, those who are incompletely vaccinated and those with persistent fever despite receipt of anti-malarials may benefit from empiric antibiotic therapy or targeted microbiologic assessment to guide pathogen directed treatment. Additionally, factors associated with increased risk of bacteremia suggest delayed

access to, or poor engagement with, the health care system, and inappropriate home treatment of bacteremic children with antimalarials.

Practical and policy implications of the study

If confirmed by other studies, findings from this study may lead to new strategies to reduce morbidity and mortality. These include formulating programs focusing on rational use of malaria and antibiotics for effective management of febrile children in these settings. These findings will also inform educational programs to influence care seeking for children with fever, particularly among children with parents with low levels of education. Our findings have the potential to improve the rational use of antimalarials and antimicrobials, and thus reduce costs and drug resistance. Our findings suggest that clinicians should be discrete in prescribing antibiotics to people children presenting with fever because most of them may not have bacterial infections. The finding that in a malaria endemic region bacteremia was thrice as common in children without malaria parasitemia as in children with malaria suggests that a high index of suspicion for bacteremia, particularly NTS is required among severely ill febrile children with a negative malaria test.

Further research or directions

There is need for more studies of non-malarial fever among children residing Kisii, Homa Bay and similar settings. The importance of such studies is highlighted by the finding that over two-thirds of the children in the study had fever that was due to causes other than malaria or bacteremia. Such research will also improve our understanding of the spectrum of the pathogens causing such fevers. Reports recently emerging from SSA indicate that previously under-recognized animal-associated bacterial pathogens as well as viral infections have emerged as important causes of febrile illness among African children with substantial public health implications[5-8]. However these studies used highly sensitive techniques such as

polymerase chain reaction (PCR) methods or serology that were not available in our study. There is need for collaborative research in different geographical, and malaria endemic settings involving a network of reference of laboratories in order to expand laboratory evaluations to include a wide range of emerging infectious agents causing febrile illness among African children. Such evaluations should include blood cultures, serologic, and molecular evaluations as recently demonstrated[7]. Besides, future research should include matched controls of children without fever so as to allow the clarification of the clinical significance of the pathogens, as well as the estimations of attributable fractions for various infectious agents in order to inform prevention priorities.

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