The Relationship Between Invasive Nontyphoidal *Salmonella* Disease, Other Bacterial Bloodstream Infections, and Malaria in Sub-Saharan Africa

Se Eun Park,[1] Gi Deok Pak,[1] Peter Aaby,[2,3] Yaw Adu-Sarkodie,[4,5] Mohammad Ali,[1,6] Abraham Aseffa,[7] Holly M. Biggs,[8,9] Morten Bjerregaard-Andersen,[2,3] Robert F. Breiman,[10,11] John A. Crump,[8,9,12,13] Ligia Maria Cruz Espinoza,[1] Muna Ahmed Eltayeb,[14] Nagla Gasmelseed,[14] Julian T. Hertz,[8,9] Justin Im,[1] Anna Jaeger,[15] Leon Parfait Kabore,[16] Vera von Kalckreuth,[1] Karen H. Keddy,[17,18] Frank Konings,[1] Ralf Krumkamp,[15] Calman A. MacLennan,[19,20] Christian G. Meyer,[21] Joel M. Montgomery,[10] Aissatou Ahmet Niang,[22] Chelsea Nichols,[1] Beatrice Olack,[23] Ursula Panzner,[1] Jin Kyung Park,[1] Henintsoa Rabezanahary,[24] Raphaël Rakotozandrindrainy,[24] Emmanuel Sampo,[16,25] Nimako Sarpong,[4] Heidi Schütt-Gerowitt,[1,26] Arvinda Sooka,[17] Abdramane Bassiahi Soura,[25] Amy Gassama Sow,[22,27] Adama Tall,[22] Mekonnen Teferi,[7] Biruk Yeshitela,[7] Jürgen May,[15] Thomas F. Wierzba,[1] John D. Clemens,[1,28,29] Stephen Baker,[30] and Florian Marks[1]

[1]International Vaccine Institute, Seoul, Republic of Korea; [2]Bandim Health Project, Bissau, Guinea-Bissau; [3]Research Center for Vitamins and Vaccines, Copenhagen, Denmark; [4]Kumasi Centre for Collaborative Research in Tropical Medicine, and [5]School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; [6]Johns Hopkins University, Baltimore, Maryland; [7]Armauer Hansen Research Institute, Addis Ababa, Ethiopia; [8]Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, North Carolina; [9]Kilimanjaro Christian Medical Centre, Moshi, Tanzania; [10]Centers for Disease Control and Prevention, Nairobi, Kenya; [11]Emory Global Health Institute, Emory University, Atlanta, Georgia; [12]Duke Global Health Institute, Duke University, Durham, North Carolina; [13]Centre for International Health, University of Otago, Dunedin, New Zealand; [14]University of Gezira, Wad Medani, Sudan; [15]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; [16]Schiphra Hospital, Ouagadougou, Burkina Faso; [17]National Institute for Communicable Diseases, and [18]Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [19]Jenner Institute, Nuffield Department of Medicine, University of Oxford, and [20]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom; [21]Institute of Tropical Medicine, Eberhard-Karls University Tübingen, Germany; [22]Institute Pasteur Senegal, Dakar; [23]Kenya Medical Research Institute (KEMRI), Nairobi; [24]University of Antananarivo, Madagascar; [25]Institut Supérieur des Sciences de la Population, University of Ouagadougou, Burkina Faso; [26]Institute of Medical Microbiology, University of Cologne, Germany; [27]Université Cheikh Anta Diop de Dakar, Senegal; [28]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka; [29]Fielding School of Public Health, University of California, Los Angeles; and [30]Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Background. Country-specific studies in Africa have indicated that *Plasmodium falciparum* is associated with invasive nontyphoidal *Salmonella* (iNTS) disease. We conducted a multicenter study in 13 sites in Burkina Faso, Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, South Africa, Sudan, and Tanzania to investigate the relationship between the occurrence of iNTS disease, other systemic bacterial infections, and malaria.

Methods. Febrile patients received a blood culture and a malaria test. Isolated bacteria underwent antimicrobial susceptibility testing, and the association between iNTS disease and malaria was assessed.

Results. A positive correlation between frequency proportions of malaria and iNTS was observed ($P = .01$; $r = 0.70$). Areas with higher burden of malaria exhibited higher odds of iNTS disease compared to other bacterial infections (odds ratio [OR], 4.89; 95% CI, 1.61–14.90; $P = .005$) than areas with lower malaria burden. Malaria parasite positivity was associated with iNTS disease (OR, 2.44; $P = .031$) and gram-positive bacteremias, particularly *Staphylococcus aureus*, exhibited a high proportion of coinfection with *Plasmodium* malaria. *Salmonella* Typhimurium and *Salmonella* Enteritidis were the predominant NTS serovars (53/73; 73%). Both moderate (OR, 6.05; $P = .0001$) and severe (OR, 14.62; $P < .0001$) anemia were associated with iNTS disease.

Conclusions. A positive correlation between iNTS disease and malaria endemicity, and the association between *Plasmodium* parasite positivity and iNTS disease across sub-Saharan Africa, indicates the necessity to consider iNTS as a major cause of febrile illness in malaria-holoendemic areas. Prevention of iNTS disease through iNTS vaccines for areas of high malaria endemicity, targeting high-risk groups for *Plasmodium* parasitic infection, should be considered.

Keywords. *Salmonella*; NTS; invasive NTS; *Plasmodium*; malaria.

Nontyphoidal *Salmonella* (NTS) infections in specific populations in sub-Saharan Africa can result in invasive disease. Invasive NTS (iNTS) disease is associated with diverse, nonspecific clinical features that can be difficult to distinguish from typhoid fever and other febrile illnesses [1, 2]. There is a paucity of data regarding the incidence of iNTS disease in Africa, but a recent systematic review of the global burden of iNTS disease estimated 3.4 million cases in 2010 with the highest burden of disease arising in Africa, with a case fatality rate of 20% [3, 4]. Multiple studies have been conducted to investigate host-associated risk factors for iNTS disease and have identified recent malaria [5], human immunodeficiency virus (HIV) [6], severe anemia [7], severe malnutrition [8], and sickle cell disease [9, 10] to be associated. However, our understanding of the natural history of iNTS, the transmission dynamics of the organisms, the features of asymptomatic human infections, the reservoirs, and the immune responses during infection are incomplete.

Among the 198 million global cases of malaria in 2013, 584 000 deaths were reported, 90% of which originated from sub-Saharan Africa [11]. The burden of malaria correlates with the force of infection, with disease concentrated among infants and young children in high-transmission areas [12]. Yet, the misdiagnosis of bacteremia as malaria is frequent, especially in sites with inadequate microbiological laboratory infrastructure to identify the pathogenic microorganisms [13]. Where transmission is intense, seemingly healthy older children and adults may experience *Plasmodium falciparum* infection with partial immunity, which may prevent the development of clinical symptoms [14]. This, coupled with the nonspecific clinical presentation of iNTS disease [2], means that patients with iNTS disease may be treated with antimalarials and/or inappropriate antimicrobials. Growing drug-resistant malaria [15] and antimicrobial resistance among iNTS strains implies that empiric antimicrobial regimens may not be effective against the locally circulating organisms [16, 17].

Although the relationship between iNTS disease and malaria has been previously investigated [5, 18], those studies have been constrained by the use of noncomparable study designs and by being restricted to one site. Here we describe the epidemiological features of iNTS disease and malaria in comparison to other community-acquired bacteremias in sub-Saharan Africa using a standardized multicenter approach.

METHODS

Febrile Disease Surveillance

Surveillance of invasive *Salmonella* infections in sub-Saharan Africa was conducted as described elsewhere (Marks F. et al, unpublished data), [19–20]. In brief, standardized healthcare-facility-based surveillance of febrile patients was instituted in 13 sites in sub-Saharan Africa: Nioko and Polesgo, Burkina Faso (BF); Butajira, Ethiopia; Asante Akim North District (AAN), Ghana; Bissau, Guinea-Bissau; the Kibera informal settlement in Nairobi, Kenya; Imerintsiatosika and Isotry, Madagascar; Pikine, Senegal; Pietermaritzburg, South Africa; East Wad Medani (EWM), Sudan; and Moshi Urban and Moshi Rural, Tanzania.

Study Population, Inclusion Criteria, Sample Collection, and Laboratory Diagnoses

Febrile patients from all age groups (except in Ghana, where only children aged <15 years were enrolled) with a tympanic or axillary temperature of ≥38.0°C or ≥37.5°C, respectively, living in the defined study areas were eligible for recruitment. For inpatients, reported fever within the period of 72 hours prior to admission was added to the inclusion criteria (Marks F. et al, unpublished data), [19]. Blood specimens were obtained from febrile patients for aerobic blood culturing (BD Bactec, Becton-Dickinson, Franklin Lakes, New Jersey or BacT/ALERT, bioMérieux, Marcy l'Etoile, France) and malaria diagnostics were performed via a combination of thick and thin blood smears and rapid malaria tests (SD Bioline Malaria Ag

P.f/P.v, SD Standard Diagnostics, Suwon, Republic of Korea) that varied among sites.

Data Analysis

As the adjusted incidence rates of bacteremia calculated per 100 000 person-years of observation are described elsewhere (Marks F. et al, unpublished data), here, frequency proportions of bacteremias and *Plasmodium* parasite positivity are presented. After a normality test of our dataset, correlations between malaria and bacteremias from all sites were analyzed with the Spearman correlation coefficient (*r*). To measure the odds ratios (ORs) of iNTS infections per exposure to malaria endemicity (proportion of malaria frequencies at health facilities used as a proxy), generalized estimating equation (GEE) logistic regression was used. Stratified logistic regression for the univariate and multivariable analysis was performed to understand the individual-level covariates including *Plasmodium spp.* parasite positivity and anemia, considered to be associated with iNTS disease. Data were statistically analyzed taking into account age groups, sex, site-specific characteristics, and clinical data using Microsoft Excel (version 9.0, Microsoft Corporation, Redmond, Washington) and SAS software (version 9.4, SAS Institute, Cary, North Carolina).

RESULTS

Site and Age-Stratified Frequencies of iNTS Disease, Other Bacteremias, Malaria, and Coinfections

A total of 13 431 febrile patients were enrolled and had a blood culture performed during 2010–2014. Of the 13 431 blood cultures, 497 (4%) were positive for a bacterial pathogen: 73 (15%) NTS (*Salmonella* Typhimurium [n = 38/73; 52%], *Salmonella* Enteritidis [n = 15/73; 21%], *Salmonella* Dublin [n = 10/73; 14%], and several other undetermined *Salmonella* serovars [n = 10/73; 14%]); 3 (1%) *Salmonella* Paratyphi A; 137 (28%) *Salmonella* Typhi; and 272 (55%) non-*Salmonella* bacteremias (179/272 [66%] gram-positive and 93/272 [34%] non-*Salmonella* gram-negative bacteremias). The proportion of NTS among positive blood cultures was the highest in Nioko, BF (8/27; 30%) and AAN, Ghana (46/157; 29%), although the proportion of gram-positive bacteremia was equivalently high in the latter (Figure 1).

A total of 11 421 of 13 431 (85%) blood samples were screened for malaria, of which 3133 (27%) were positive by any malaria test. The proportion of malaria frequencies presented at healthcare facilities was the highest in Polesgo, BF (444/744; 60%) and Nioko, BF (430/908; 47%), followed by AAN, Ghana (1139/2651; 43%), and EWM, Sudan (254/632; 40%). Sites in Ethiopia, Madagascar, and Tanzania had the lowest proportion of malaria-positive results. Of 3133 positive malaria tests, 1386 (44%) were from children aged <5 years. All, with the exception of 20 *Plasmodium malariae* and 2 *Plasmodium ovale* infections in AAN, Ghana and with 332 missing data on the *Plasmodium* species, were *P. falciparum* monoinfections.

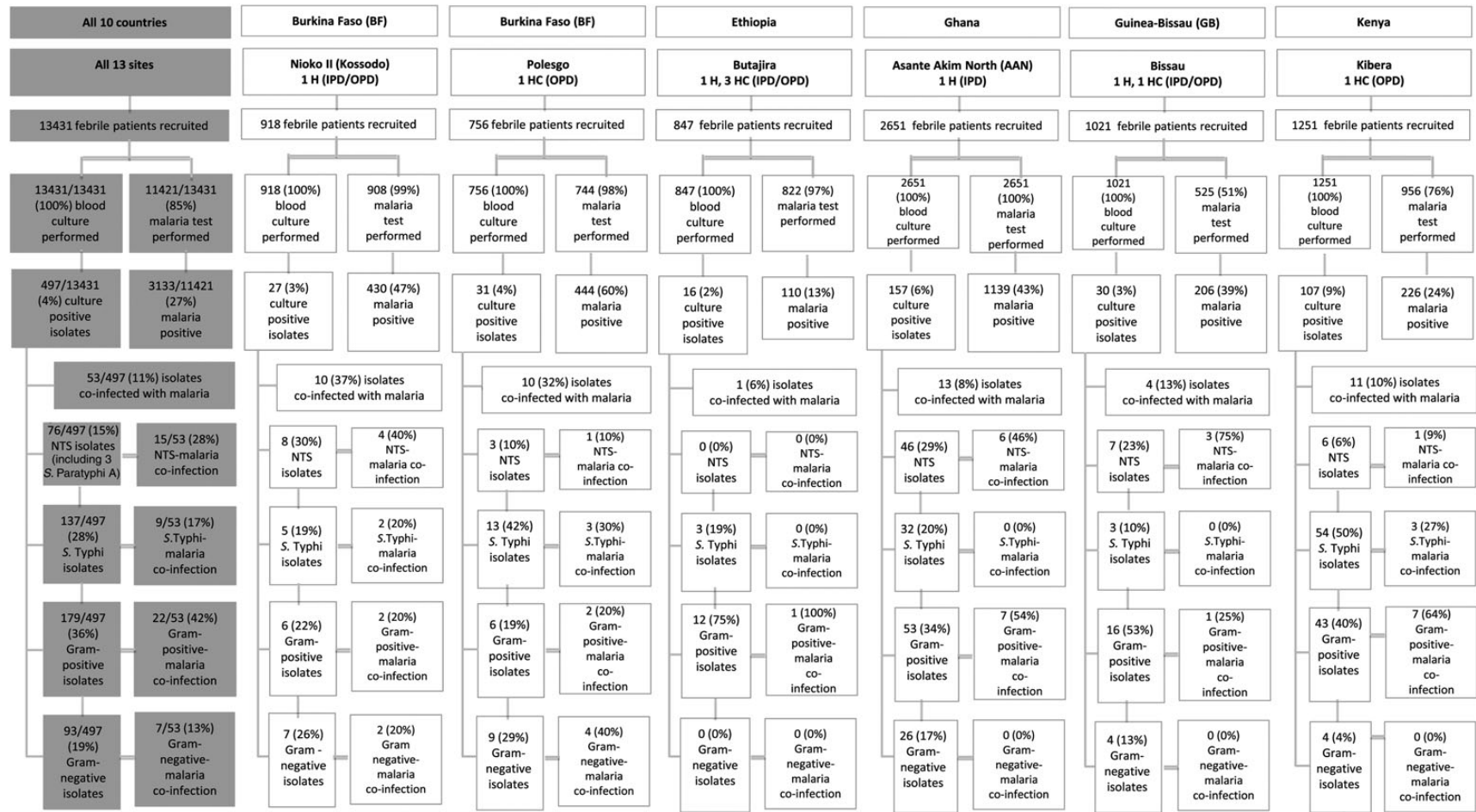The proportion of malaria coinfection with any bacterial pathogen from all 13 sites was 11% (53/497), predominantly

Figure 1. Flowchart of Typhoid Fever Surveillance in Africa Program (TSAP) blood culture and malaria test performance. Blood culture–positive isolates: contaminants excluded (Marks F. et al, unpublished data). Gram-negative isolates: *Salmonella* species excluded. Malaria tests performed per site: Nioko, Burkina Faso (BF; thick/thin blood smears), Polesgo, BF (thick/thin blood smears), Butajira, Ethiopia (Rapid Diagnostic Test [RDT] and thick/thin blood smears), Asante Akim North District (AAN), Ghana (thick/thin blood smears), Bissau, Guinea-Bissau (GB; thick/thin blood smears), Kibera, Kenya (thick blood smear), Imerintsiatosika, Madagascar (MG; RDT and thick/thin blood smears), Isotry, MG (RDT and thick/thin blood smears), Pikine, Senegal (RDT if available and thick/thin blood smears), Pietermaritzburg, South Africa (SA; malaria-free zone: no malaria test performed), East Wad Medani (EWM), Sudan (thick/thin blood smears), Moshi Rural (MR), Tanzania (thick/thin blood smears), and Moshi Urban (MU), Tanzania (thick/thin blood smears). Numerators and denominators for each percentages (%) in the flowchart (in descending order from top to bottom): % of blood culture performed of recruited patients; % of malaria test performed of recruited patients; % of blood culture positive isolates of blood culture performed; % of malaria parasite–positive cases of malaria test performed; % of bacterial isolates
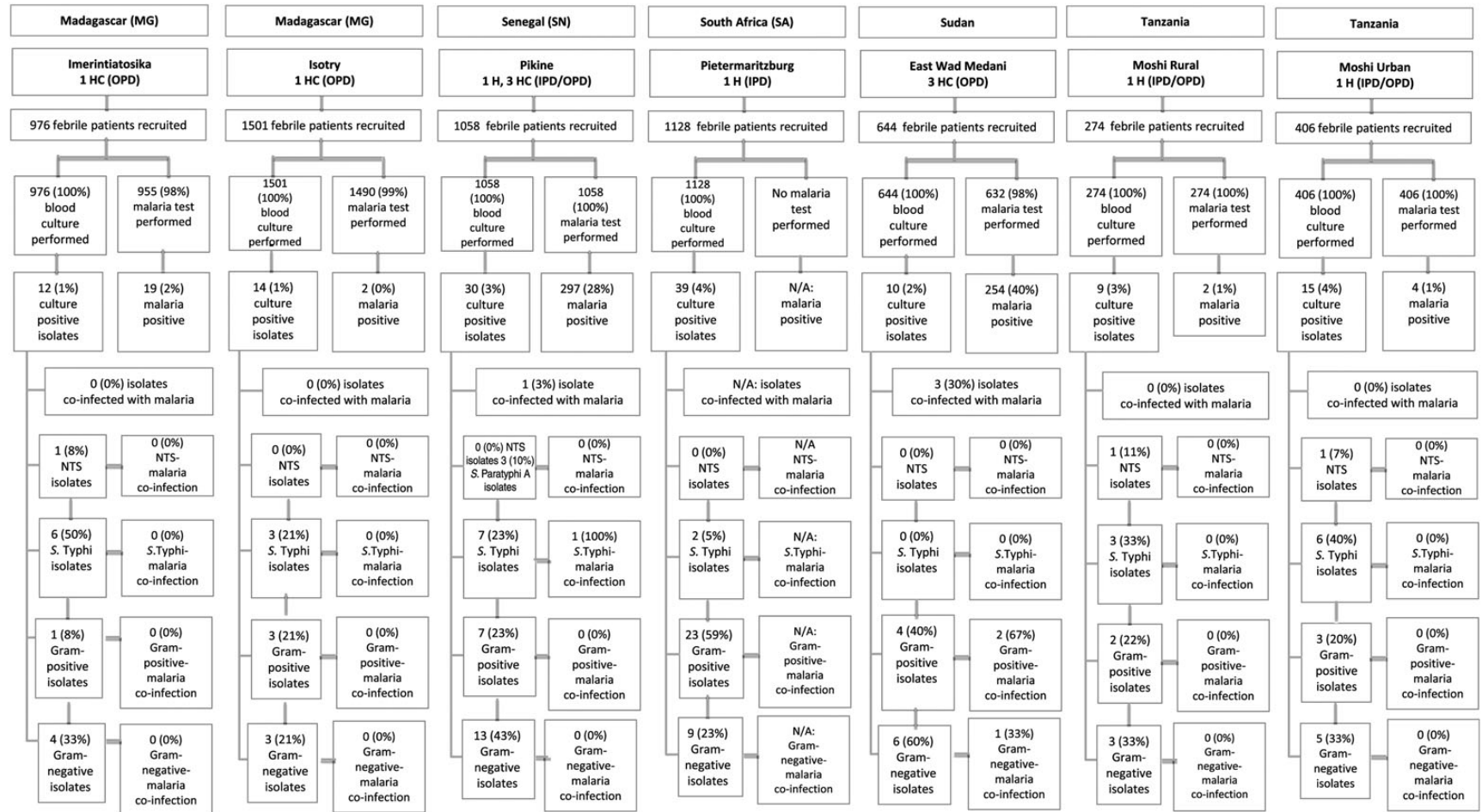
Madagascar (MG)	Madagascar (MG)	Senegal (SN)	South Africa (SA)	Sudan	Tanzania	Tanzania
Imerintiatosika 1 HC (OPD)	Isotry 1 HC (OPD)	Pikine 1 H, 3 HC (IPD/OPD)	Pietermaritzburg 1 H (IPD)	East Wad Medani 3 HC (OPD)	Moshi Rural 1 H (IPD/OPD)	Moshi Urban 1 H (IPD/OPD)
976 febrile patients recruited	1501 febrile patients recruited	1058 febrile patients recruited	1128 febrile patients recruited	644 febrile patients recruited	274 febrile patients recruited	406 febrile patients recruited

Imerintiatosika (MG): 976 (100%) blood culture performed; 955 (98%) malaria test performed; 12 (1%) culture positive isolates; 19 (2%) malaria positive; 0 (0%) isolates co-infected with malaria; 1 (8%) NTS isolates, 0 (0%) NTS-malaria co-infection; 6 (50%) S. Typhi isolates, 0 (0%) S.Typhi-malaria co-infection; 1 (8%) Gram-positive isolates, 0 (0%) Gram-positive-malaria co-infection; 4 (33%) Gram-negative isolates, 0 (0%) Gram-negative-malaria co-infection.

Isotry (MG): 1501 (100%) blood culture performed; 1490 (99%) malaria test performed; 14 (1%) culture positive isolates; 2 (0%) malaria positive; 0 (0%) isolates co-infected with malaria; 0 (0%) NTS isolates, 0 (0%) NTS-malaria co-infection; 3 (21%) S. Typhi isolates, 0 (0%) S.Typhi-malaria co-infection; 3 (21%) Gram-positive isolates, 0 (0%) Gram-positive-malaria co-infection; 3 (21%) Gram-negative isolates, 0 (0%) Gram-negative-malaria co-infection.

Pikine (SN): 1058 (100%) blood culture performed; 1058 (100%) malaria test performed; 30 (3%) culture positive isolates; 297 (28%) malaria positive; 1 (3%) isolate co-infected with malaria; 0 (0%) NTS isolates 3 (10%) S. Paratyphi A isolates, 0 (0%) NTS-malaria co-infection; 7 (23%) S. Typhi isolates, 1 (100%) S.Typhi-malaria co-infection; 7 (23%) Gram-positive isolates, 0 (0%) Gram-positive-malaria co-infection; 13 (43%) Gram-negative isolates, 0 (0%) Gram-negative-malaria co-infection.

Pietermaritzburg (SA): 1128 (100%) blood culture performed; No malaria test performed; 39 (4%) culture positive isolates; N/A: malaria positive; N/A: isolates co-infected with malaria; 0 (0%) NTS isolates, N/A NTS-malaria co-infection; 2 (5%) S. Typhi isolates, N/A S.Typhi-malaria co-infection; 23 (59%) Gram-positive isolates, N/A Gram-positive-malaria co-infection; 9 (23%) Gram-negative isolates, N/A Gram-negative-malaria co-infection.

East Wad Medani (Sudan): 644 (100%) blood culture performed; 632 (98%) malaria test performed; 10 (2%) culture positive isolates; 254 (40%) malaria positive; 3 (30%) isolates co-infected with malaria; 0 (0%) NTS isolates, 0 (0%) NTS-malaria co-infection; 0 (0%) S. Typhi isolates, 0 (0%) S.Typhi-malaria co-infection; 4 (40%) Gram-positive isolates, 2 (67%) Gram-positive-malaria co-infection; 6 (60%) Gram-negative isolates, 1 (33%) Gram-negative-malaria co-infection.

Moshi Rural (Tanzania): 274 (100%) blood culture performed; 274 (100%) malaria test performed; 9 (3%) culture positive isolates; 2 (1%) malaria positive; 0 (0%) isolates co-infected with malaria; 1 (11%) NTS isolates, 0 (0%) NTS-malaria co-infection; 3 (33%) S. Typhi isolates, 0 (0%) S.Typhi-malaria co-infection; 2 (22%) Gram-positive isolates, 0 (0%) Gram-positive-malaria co-infection; 3 (33%) Gram-negative isolates, 0 (0%) Gram-negative-malaria co-infection.

Moshi Urban (Tanzania): 406 (100%) blood culture performed; 406 (100%) malaria test performed; 15 (4%) culture positive isolates; 4 (1%) malaria positive; 0 (0%) isolates co-infected with malaria; 1 (7%) NTS isolates, 0 (0%) NTS-malaria co-infection; 6 (40%) S. Typhi isolates, 0 (0%) S.Typhi-malaria co-infection; 3 (20%) Gram-positive isolates, 0 (0%) Gram-positive-malaria co-infection; 5 (33%) Gram-negative isolates, 0 (0%) Gram-negative-malaria co-infection.

Figure 1 continued. coinfected with malaria of blood culture-confirmed positive isolates; % of nontyphoidal *Salmonella* (NTS), *S.* Typhi, Gram-positive, and non-*Salmonella* gram-negative isolates of blood culture-confirmed positive isolates; % of isolates with NTS-malaria coinfection, *S.* Typhi-malaria coinfection, gram-positive-malaria coinfection, non-*Salmonella* gram-negative-malaria coinfection of all bacterial isolates coinfected with malaria. Malaria species: 2801/3133 malaria positives confirmed with malaria species; 332/3133 missing data on malaria species; 2779/2801 *Plasmodium falciparum*; 20/2801 *Plasmodium malariae*; 2/2801 *Plasmodium ovale*. Malaria species confirmed per site listed below. Nioko, BF: 430/430 *P. falciparum*; Polesgo, BF: 444/444 *P. falciparum*. Butajira, Ethiopia: 26/110 *P. falciparum* and 84 missing data on malaria species. AAN, Ghana: 1111/1139 *P. falciparum*, 20/1139 *P. malariae*, 2/1139 *P. ovale*, and 6 missing data on malaria species. Bissau, GB: 196/206 *P. falciparum* and 10 missing data on malaria species. Kibera, Kenya: not available/226 (no data on malaria species). Imerintsiatosika, MG: 19/19 *P. falciparum*; Isotry, MG: 2/2 *P. falciparum*. Pikine, Senegal: 291/297 *P. falciparum*, and 6 missing data on malaria species. Pietermaritzburg, SA: No malaria test performed as the country is malaria-free. EWM, Sudan: 254/254 *P. falciparum*. MR, Tanzania: 2/2 *P. falciparum*; MU, Tanzania: 4/4 *P. falciparum*. Abbreviations: H, hospital; HC, health center; IPD, inpatient department; NA, not available; OPD, outpatient department.

Table 1. Age-Specific Frequencies of Organisms Isolated From Blood Culture and Malaria Test

Blood Culture–Confirmed Organism	All Ages	0 to <2 y	2 to <5 y	5 to <15 y	≥15 y	Median Age, y (Q1–Q3)
Blood culture performed (No.)	13 431	2843	2402	3355	4831	
All pathogenic bacteria (no., % of No.)	497 (4)	126 (4)	107 (4)	141 (4)	123 (3)	5 (1–14)
NTS including 3 *S.* Paratyphi A (no., % of all pathogenic bacteria)[a]	76 (15)	35 (28)	24 (22)	10 (7)	7 (6)	2 (1–3)
Salmonella Typhi (no., % of all pathogenic bacteria)	137 (28)	6 (5)	34 (32)	67 (48)	30 (24)	8 (4–14)
Gram-negative bacteria (no., % of all pathogenic bacteria)[b]	93 (19)	34 (27)	6 (6)	15 (11)	38 (31)	6 (2–14)
Gram-positive bacteria (no., % of all pathogenic bacteria)	179 (36)	50 (40)	42 (39)	47 (33)	40 (33)	4 (1–13)
Malaria test performed (No.)[c]	11421	2136	2031	3007	4247	
Malaria positive (no., % of No.)	3133 (27)	527 (25)	859 (42)	1083 (36)	664 (16)	6 (2–12)
Plasmodium falciparum (no., % of malaria positive)	2779 (89)	500 (95)	782 (91)	913 (84)	584 (88)	5 (2–12)
Plasmodium malariae (no., % of malaria positive)	20 (1)	5 (1)	8 (1)	7 (1)	0 (0)	4 (2–6)
Plasmodium ovale (no., % of malaria positive)	2 (0.1)	0 (0)	0 (0)	2 (0.2)	0 (0)	6 (6–6)
Malarial-bacterial coinfection (no., % of all pathogenic bacteremia)	53 (11)	12 (10)	8 (7)	22 (16)	11 (9)	6 (2–11)
Malaria-NTS (no., % of coinfection)	15 (28)	7 (58)	4 (50)	4 (18)	0 (0)	2 (1–5)
Malaria-*S.* Typhi (no., % of coinfection)	9 (17)	0 (0)	0 (0)	5 (23)	4 (36)	9 (6–22)
Malaria gram-negative (no., % of coinfection)[d]	7 (13)	1 (8)	1 (13)	2 (9)	3 (27)	5 (1–11)
Malaria gram-positive (no., % of coinfection)[e]	22 (42)	4 (33)	3 (38)	11 (50)	4 (36)	7 (3–11)

Abbreviation: NTS, nontyphoidal *Salmonella*.

[a] Total of 3 *S.* Paratyphi A and 73 NTS isolates: 38 of 73 (50%) *Salmonella* Typhimurium, 15 of 73 (20%) *Salmonella* Enteritidis, 10 of 73 (14%) *Salmonella* Dublin, and 10 of 73 (14%) other undetermined *Salmonella* serovars. The 3 *S.* Paratyphi A isolates were all female; 14, 20, and 25 years of age; none exhibited malaria coinfection.

[b] Gram-negative bacteria: *Salmonella* species excluded.

[c] Malaria test was performed through Rapid Diagnostic Test or/and blood film (thick and thin smears) (Figure 1). Of 11 421 blood samples screened for malaria, 3133 (27%) resulted positive: 20 *P. malariae* and 2 *P. ovale* were from Asante Akim North, Ghana. Three hundred thirty-two malaria-positive cases missed data on malaria species. Except for these, all were *P. falciparum.*

[d] Malaria gram-negative coinfection (*Salmonella* species excluded): Of the 7 cases of malaria coinfection with gram-negative bacteria, 3 were *Escherichia coli* (all >15 years of age) and 2 *Pseudomonas aeruginosa* (one <2 years of age, one 5 to <15 years of age). No cases of malaria coinfection with *Klebsiella pneumoniae.*

[e] Malaria gram-positive coinfection: Of 22 cases of malaria coinfection with gram-positive bacteria, 59% (13/22) were *Staphylococcus aureus* (3 between 2 and <5 years, 6 between 5 and <15 years, and 4 aged ≥15 years) and 5 *Streptococcus pneumoniae* (2 under 2 years of age, 3 between 5 and <15 years of age).

gram-positive bacterial coinfection with malaria (22/53; 42%) and NTS-malaria coinfection (15/53; 28%) (Figure 1; Table 1). The malaria/iNTS coinfections were more common among children <5 years of age (11/20; 55%), particularly under 2 years of age (7/12; 58%), whereas all malaria/*Salmonella* Typhi coinfections occurred in children >5 years of age. Children between 5 and 15 years of age had the highest proportion of malaria/bacteremia coinfection with a gram-positive bacterium (11/22; 50%). Children <2 years of age showed the highest proportion of iNTS (35/126; 28% or 35/73; 48%) and gram-positive bacterial infections (50/126; 40% or 50/179; 28%) compared with the other age groups (Table 1).

iNTS–Malaria Correlations, Risk Factors, and Clinical Characteristics

A strong linear correlation of malaria with iNTS across sites was found ($P = .01$; $r = 0.70$) (Figure 2). Areas with higher malaria burden had higher odds of iNTS disease compared with other bacterial infections (OR, 4.89; 95% confidence interval [CI], 1.61–14.90; $P = .005$; GEE logistic regression) than areas with lower burden of malaria (Table 2). Furthermore, relative to non-NTS invasive bacterial infections, *Plasmodium* parasite positivity was associated with iNTS disease (OR, 2.44; 95% CI, 1.09–5.47; $P = .031$; stratified univariate analysis; Table 3).

Patients with severe anemia (hemoglobin level <7 g/dL for age group 0 to <5 years; <8 g/dL for ≥5 years) had >10 times higher odds of iNTS disease (univariate analysis: OR, 13.10;

$P < .0001$; multivariable analysis: OR, 14.62; $P < .0001$) than non-NTS bacteremia. Moderate anemia was also associated with iNTS disease (Table 3). Among 76 iNTS patients, which includes 3 *S.* Paratyphi A cases, 34 (45%) had severe anemia and 10 (15%) iNTS patients <10 years of age were severely underweight. iNTS patients exhibited the highest proportion of hospitalizations (54/76; 71%) and diarrhea (22/76; 29%) compared with other bacteremias: 32% (44/137) and 24% (33/137) of patients infected with *Salmonella* Typhi; 54% (96/179) and 16% (29/179) of patients with gram-positive bacterial infections; and 56% (52/93) and 16% (15/93) of patients with non-*Salmonella* gram-negative bacterial infections were hospitalized and had diarrhea.

DISCUSSION

In this study, we demonstrated a positive malaria-iNTS correlation over multiple countries in sub-Saharan Africa. Further, iNTS disease and gram-positive bacteremia were the most common malarial/bacterial coinfections in these areas, exceeding the number of coinfections with *Salmonella* Typhi and non-*Salmonella* gram-negative bacteremia. However, some of the sites (EWM, Sudan and Kibera, Kenya) did not follow this trend, as they had a low level of iNTS infections despite a high burden of malaria. This could be due to low local levels of transmission of iNTS disease, leading to reduced exposure
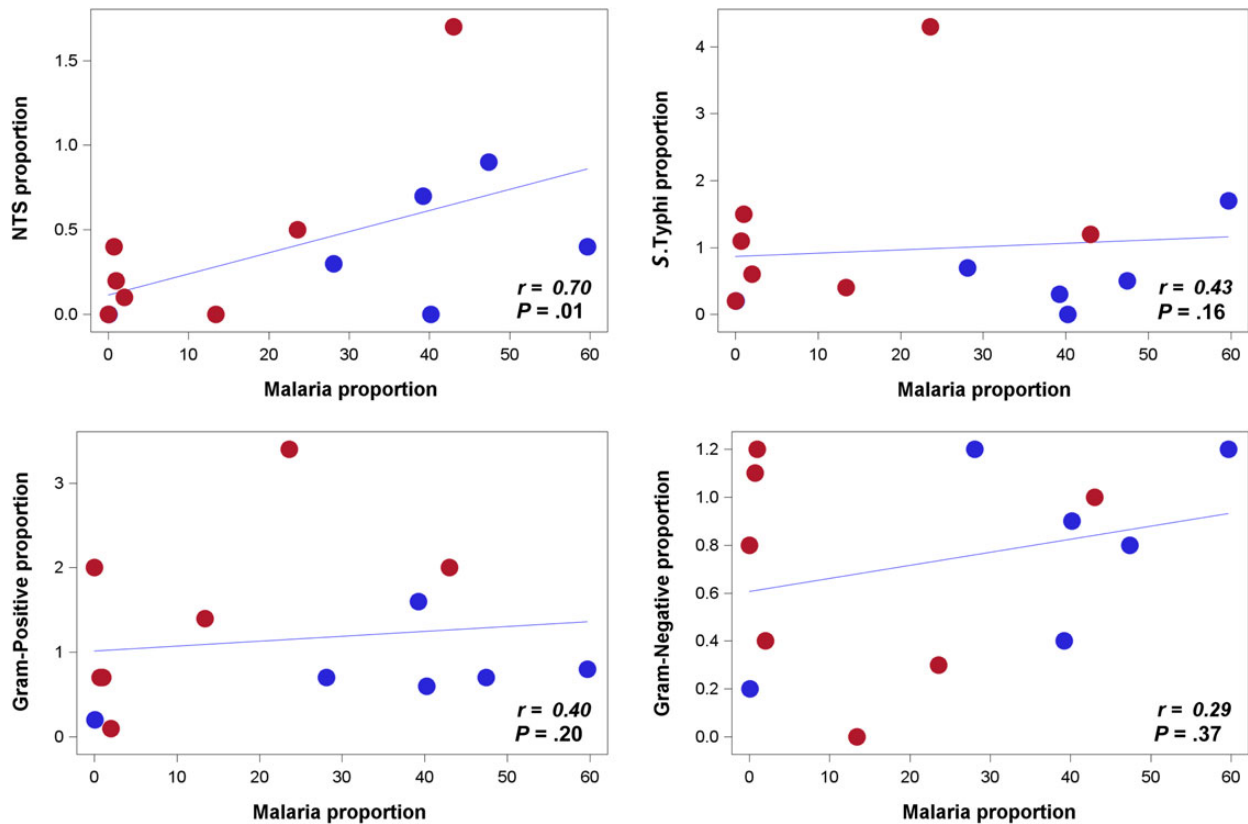
Figure 2. Correlation between frequency proportions of malaria and invasive nontyphoidal *Salmonella* (iNTS) compared to malaria with other invasive bacteria across 13 sites. Spearman correlation coefficient (*r*) is analyzed based on the proportion of frequencies of malaria, iNTS, *Salmonella* Typhi, gram-positive and non-*Salmonella* gram-negative bacterial isolates per site, controlled by each site study period. (Proportions of frequencies are calculated as iNTS, *S.* Typhi, gram-positive bacteria, non-*Salmonella* gram-negative bacteria positivity out of blood culture performed; malaria positivity out of malaria test performed.) Scattered dots represent each study sites. Frequencies of bacteremias and *Plasmodium* parasite positivity per site are noted in Figure 1. Thirteen sites exhibited in the scattered dots are grouped per surveillance period below or above the median duration of surveillance period (20 months). Sites colored in red dots are those with surveillance period of >20 months: Ethiopia, Ghana, Kenya, South Africa, and Tanzania (urban and rural). Sites colored in blue dots are those with a surveillance period of ≤20 months: Burkina Faso (Nioko and Polesgo), Guinea-Bissau, Madagascar (Imerintsiatosika and Isotry), Senegal, and Sudan. Some sites with same or similar values may appear overlapped, but all 13 sites are dotted in the plot. 3 *S.* Paratyphi A isolates from Senegal are grouped under NTS in this analysis.

of children with malaria to iNTS strains. The patterns of iNTS and malaria infections should also be further investigated in the context of the travel history of Kibera residents or rural–urban migration to Kibera, as people residing in Kibera travel within Kenya and their malaria infections may be associated with travel to areas with a greater malaria risk [21].

Our data support other studies [22–24] that suggest an elevated association between iNTS disease and *Plasmodium* parasite–positive individuals. We additionally found a high proportion of gram-positive bacterial coinfections with malaria, contrasting with earlier studies from the Gambia [5] and Malawi [25], that showed a strong association between malaria and iNTS disease, but little evidence for any association between malaria and non-*Salmonella* bacteremias. However, a recent meta-analysis of studies of children with severe malaria and bacteremia [26] indicated that in 5 studies, the overall prevalence of gram-positive organisms in children with malaria was greater than that of gram-negative organisms with *Staphylococcus aureus*,

which was the main pathogen isolated in 3 studies. This observation is consistent with malaria-induced impairment of neutrophil function as an underlying causal link: Patients with chronic granulomatous disease, a congenital defect of neutrophil oxidative burst, are particularly susceptible to invasive *S. aureus* and *Salmonella* infections [27].

Although further investigation is required to understand the temporal precedence of infections with malaria parasites and NTS for a risk factor analysis for iNTS disease (including the association between iNTS disease and recent malaria vis-à-vis current malaria or malaria parasite density) [22, 23, 28], a multitude of potential mechanisms may explain this observation. Malaria impairs the oxidative burst function of macrophages and neutrophils, which is likely to increase the risk for iNTS disease, as oxidative burst is key for eliminating *Salmonella* from the intracellular niches [29–32]. More recently, the association has been linked to induction of heme oxygenase-1 (HO-1) in a mouse model of malaria that made mice more susceptible to

Table 2. Characteristics of Bacterial Infection per Malaria Endemicity

Characteristic	Sites With Lower Malaria Endemicity[a] (n[c] = 105)	Sites With Higher Malaria Endemicity[b] (n = 392)	Odds Ratio (95% CI)	P Value
iNTS infection[d]	3 (2.9)	73 (18.6)	4.89 (1.61–14.90)	.005
Salmonella Typhi infection	23 (21.9)	114 (29.1)	0.94 (.38–2.32)	.893
Gram-positive bacterial infection	44 (41.9)	135 (34.4)	0.76 (.31–1.84)	.540
Gram-negative bacterial infection	24 (22.9)	69 (17.6)	1.09 (.48–2.46)	.842
Malaria coinfection with all bacteremia[e]	1 (1.5)	52 (14.6)	17.44 (2.76–110.10)	.002

Risk Factor (Exposure) spans the four right-hand columns.

Data are presented as No. (%) unless otherwise specified.

Abbreviations: CI, confidence interval; iNTS, invasive nontyphoidal Salmonella.

[a] Sites with lower malaria endemicity (lower-frequency proportions of malaria): Ethiopia (Butajira), Madagascar (Imerintsiatosika, Isotry), Tanzania (Moshi Rural, Moshi Urban), and malaria-free South Africa (Pietermaritzburg) (see Figure 1).

[b] Sites with higher malaria endemicity (higher-frequency proportions of malaria): Burkina Faso (Nioko, Polesgo), Ghana (Asante Akim North District), Guinea-Bissau (Bandim), Kenya (Kibera), Senegal (Pikine), and Sudan (East Wad Medani) (see Figure 1).

[c] No. = blood culture–confirmed real pathogen (excluding contaminants).

[d] iNTS infection: 3 S. Paratyphi A isolates grouped under iNTS in this analysis.

[e] Malaria coinfection with all bacteremia is analyzed based on number of blood culture positive cases with malaria test performed. Out of total 497 blood culture positive isolates, 422 had malaria test performed, which is grouped into sites with lower malaria endemicity (n = 65) and sites with higher malaria endemicity (n = 357).

NTS infection [33]. The same researchers demonstrated that impaired neutrophil oxidative burst was associated with HO-1 induction in Gambian children with malaria [34]. Malaria has also been shown to increase susceptibility to NTS infection in the mice through inhibition of interleukin 12 production [35] and increased interleukin 10 levels [36]. The precise contribution of this HO-1 pathway mechanism to enhanced susceptibility to iNTS disease in humans needs to be further investigated.

The study has some limitations. First, the surveillance period was not long enough to investigate any potential seasonality of malaria, iNTS disease, and coinfections. Second, given that individuals with mild cases of malaria do not necessarily seek healthcare at hospitals, the true burden of malaria is likely to

Table 3. Univariate and Multivariable Analyses of Characteristics Associated With Invasive Nontyphoidal Salmonella Disease[a]

Characteristics Associated With iNTS Disease	iNTS (n[b] = 76)	Non-NTS Bacteremias (n[c] = 421)	OR (95% CI)	P Value	OR (95% CI)	P Value
Malaria parasite positivity	15 (19.7)	38 (9.0)	2.44 (1.09–5.47)	.031	2.10 (.84–5.24)	.110
Nutritional status[d]						
Moderate underweight (<10 y)[e]	8 (11.9)	30 (12.0)	1.05 (.43–2.54)	.917	NA[f]	NA[f]
Severe underweight (<10 y)[g]	10 (14.9)	47 (18.9)	0.96 (.43–2.14)	.919	NA[f]	NA[f]
Anemia status[h]						
Moderate anemia[i]	27 (35.5)	65 (15.4)	6.99 (2.79–17.52)	<.0001	6.05 (2.41–15.21)	.0001
Severe anemia[j]	34 (44.7)	69 (16.4)	13.10 (5.05–33.97)	<.0001	14.62 (5.56–38.43)	<.0001

Univariate Analysis spans the iNTS, Non-NTS Bacteremias, OR (95% CI), and P Value columns. Multivariable Analysis spans the last OR (95% CI) and P Value columns.

Data are presented as No. (%) unless otherwise specified.

Abbreviations: CI, confidence interval; iNTS, invasive nontyphoidal Salmonella; NA, not applicable; NTS, nontyphoidal Salmonella; OR, odds ratio; SD, standard deviation.

[a] Stratified logistic regression was used for this univariate and multivariable analysis.

[b] Total valid case number (n) for iNTS includes the 3 S. Paratyphi A cases for this analysis.

[c] Total valid case number (n) for non-NTS bacteremia is the total number of blood culture positives (497) minus total number of NTS isolates (76 including 3 S. Paratyphi A): n = 421.

[d] Association between malnutrition (moderate and severe underweight) and iNTS disease compared to non-iNTS bacteremias. Both moderate and severe underweight cases compared with those without malnutrition.

[e] Total valid case number (No.) for moderate underweight cases for patients <10 years of age: NTS (n = 8), non-NTS bacteremia (n = 30). World Health Organization (WHO) Global Database on Child Growth and Malnutrition: z score cutoff point of < −2 SD and > −3 SD to classify moderate undernutrition.

[f] Not applicable due to missing data for patients >10 years of age.

[g] Total valid case number (No.) for severe underweight cases for patients <10 years of age: NTS (n = 10), non-NTS bacteremia (n = 47). WHO Global Database on Child Growth and Malnutrition: z score cutoff point of < −3 SD to classify severe undernutrition.

[h] Association between anemia (moderate and severe anemia) and iNTS disease compared to non-iNTS bacteremias. Both moderate and severe anemia cases compared with those without anemia.

[i] Moderate anemia: age group 0 to <5 years with hemoglobin level 7–9.9 g/dL, and age group ≥5 years with hemoglobin level 8–10.9 g/dL.

[j] Severe anemia: age group 0 to <5 years with hemoglobin level <7 g/dL, and age group ≥5 years with hemoglobin level <8 g/dL.

be underreported. Different malaria diagnostics may have also influenced the sensitivity and specificity of the results [37], and we were unable to distinguish current from recent malaria in our analysis of iNTS risk factors. Third, HIV testing was not performed, as HIV seroprevalence was not a main objective of the Typhoid Fever Surveillance in Africa Program study. Insufficient clinical data precluded the development of robust diagnostic criteria to better predict the causative agents of the patients and the cause of fever, although diarrhea was indicative of iNTS.

Our standardized multicenter approach adds additional information to the epidemiology of iNTS disease and malaria in Africa. Both the positive correlation between malaria and iNTS disease and the association between malaria positivity and iNTS disease support the role of NTS as a common etiology of invasive bacterial disease among children with malaria in areas where malaria is highly prevalent. Furthermore, given that 2 to several days may be required to confirm bacteremia microbiologically [38], patients with febrile symptoms commonly are given an antimalarial drug and antimicrobials simultaneously, rendering them prone to developing antimicrobial resistance. A similar scenario has occurred with *P. falciparum*, which is already resistant to chloroquine and sulfadoxine-pyrimethamine in most parts of sub-Saharan Africa, and potentially to artemisinin derivatives in the future [15]. A high burden of iNTS disease and malaria, coupled with increasing antimicrobial resistance [39, 40], may potentially contribute to increased severity of illness.

This provides additional evidence to prioritize the development of an iNTS vaccine, specifically designed for the African setting and used in areas of high iNTS prevalence and malaria endemicity. Such a vaccine could either be developed in a single formulation as a bivalent *Salmonella* Typhimurium and *Salmonella* Enteritidis regimen, given their predominance in sub-Saharan Africa as also found in our study, or as a trivalent compound, including *Salmonella* Typhi. Irrespective, surveillance for bacterial bloodstream infections to identify prevalent isolates should continue to monitor emerging pathogens and antimicrobial resistance. Further research on the development of rapid and accurate diagnostics of invasive *Salmonella* infections to minimize the risk of unnecessary simultaneous exposure to both antibiotics and antimalarials for those febrile patients is also warranted.

Notes

References

1. Gordon MA, Kankwatira AM, Mwafulirwa G, et al. Invasive non-typhoid salmonellae establish systemic intracellular infection in HIV-infected adults: an emerging disease pathogenesis. Clin Infect Dis 2010; 50:953–62.
2. MacLennan CA, Levine MM. Invasive nontyphoidal *Salmonella* disease in Africa: current status. Expert Rev Anti Infect Ther 2013; 11:443–6.
3. Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. Global burden of invasive nontyphoidal *Salmonella* disease. Emerg Infect Dis 2015; doi:10.3201/eid2106.140999.
4. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis 2010; 10:417–32.
5. Mabey DC, Brown A, Greenwood BM. *Plasmodium falciparum* malaria and *Salmonella* infections in Gambian children. J Infect Dis 1987; 155:1319–21.
6. Gordon MA, Banda HT, Gondwe M, et al. Non-typhoidal *Salmonella* bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. AIDS 2002; 16:1633–41.
7. Calis JC, Phiri KS, Faragher EB, et al. Severe anemia in Malawian children. N Engl J Med 2008; 358:888–99.
8. Bachou H, Tylleskär T, Kaddu-Mulindwa DH, Tumwine JK. Bacteraemia among severely malnourished children infected and uninfected with the human immunodeficiency virus-1 in Kampala, Uganda. BMC Infect Dis 2006; 6:160.
9. MacLennan CA. Host defense against malaria favors *Salmonella*. Nat Med 2012; 18:21–2.
10. Morpeth SC, Ramadhani HO, Crump JA. Invasive non-Typhi *Salmonella* disease in Africa. Clin Infect Dis 2009; 49:606–11.
11. World Health Organization. Malaria fact sheet no. 94. Geneva, Switzerland: WHO, 2014.
12. Griffin JT, Ferguson NM, Ghani AC. Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. Nat Commun 2014; 5:3136.
13. Nankabirwa J, Zurovac D, Njogu JN, et al. Malaria misdiagnosis in Uganda—implications for policy change. Malar J 2009; 8:66.
14. Marks F, Evans J, Meyer CG, et al. High prevalence of markers for sulfadoxine and pyrimethamine resistance in *Plasmodium falciparum* in the absence of drug pressure in the Ashanti region of Ghana. Antimicrob Agents Chemother 2005; 49:1101–5.
15. Plowe CV. The evolution of drug-resistant malaria. Trans R Soc Trop Med Hyg 2009; 103(suppl 1):S11–4.
16. Baker S. A return to the pre-antimicrobial era? Science 2015; 347:1064–6.
17. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive *Salmonella* disease. Vaccine 2015; 33(suppl 3):C21–9.
18. Mtove G, Amos B, Nadjm B, et al. Decreasing incidence of severe malaria and community-acquired bacteraemia among hospitalized children in Muheza, north-eastern Tanzania, 2006–2010. Malar J 2011; 10:320.
19. von Kalckreuth V, Konings F, Aaby P, et al. The Typhoid Fever Surveillance in Africa Program (TSAP): clinical, diagnostic, and epidemiological methodologies. Clin Infect Dis 2016; 62(suppl 1):S9–16.
20. Panzner U, Pak GD, Aaby P, et al. Utilization of healthcare in the Typhoid Fever Surveillance in Africa Program. Clin Infect Dis 2016; 62(suppl 1):S56–69.
21. Njuguna H, Montgomery JM, Cosmas L, et al. Malaria parasitemia among febrile patients seeking clinical care at an outpatient health facility in an urban informal settlement area in Nairobi, Kenya. Am J Trop Med Hyg 2015; doi:10.4269/ajtmh.15-0293.

22. Scott JA, Berkley JA, Mwangi I, et al. Relation between *falciparum* malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. Lancet **2011**; 378:1316–23.

23. Mackenzie G, Ceesay SJ, Hill PC, et al. A decline in the incidence of invasive nontyphoidal *Salmonella* infection in The Gambia temporally associated with a decline in malaria infection. PLoS One **2010**; 5:e10568.

24. Krumkamp R, Kreuels B, Sarpong N, et al. Association between malaria and invasive nontyphoidal *Salmonella* infection in a hospital study: accounting for Berkson's bias. Clin Infect Dis **2016**; 62(suppl 1):S83–9.

25. Bronzan RN, Taylor TE, Mwenechanya J, et al. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. J Infect Dis **2007**; 195:895–904.

26. Church J, Maitland K. Invasive bacterial co-infection in African children with *Plasmodium falciparum* malaria: a systematic review. BMC Med **2014**; 12:31.

27. Mouy R, Fischer A, Vilmer E, Seger R, Griscelli C. Incidence, severity, and prevention of infections in chronic granulomatous disease. J Pediatr **1989**; 114(4 pt 1):555–60.

28. Biggs HM, Lester R, Nadjm B, et al. Invasive *Salmonella* infections in areas of high and low malaria transmission intensity in Tanzania. Clin Infect Dis **2014**; 58:638–47.

29. Vazquez-Torres A, Jones-Carson J, Mastroeni P, Ischiropoulos H, Fang FC. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. J Exp Med **2000**; 192:227–36.

30. Winklestein JA, Marino MC, Johnston RB Jr, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine **2000**; 79:155–69.

31. Gondwe EN, Molyneux ME, Goodall M, et al. Importance of antibody and complement for oxidative burst and killing of invasive nontyphoidal *Salmonella* by blood cells in Africans. Proc Natl Acad Sci **2010**; 107:3070–5.

32. Schwarzer E, Turrini F, Ulliers D, Giribaldi G, Ginsburg H, Arese P. Impairment of macrophage functions after ingestion of *Plasmodium falciparum*-infected erythrocytes or isolated malarial pigment. J Exp Med **1992**; 176:1033–41.

33. Cunnington AJ, de Souza JB, Walther M, Riley EM. Malaria impairs resistance to *Salmonella* through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. Nat Med **2011**; 18:120–7.

34. Cunnington AJ, Njie M, Correa S, Takem EN, Riley EM, Walther M. Prolonged neutrophil dysfunction after *Plasmodium falciparum* malaria is related to hemolysis and heme oxygenase-1 induction. J Immunol **2012**; 189:5336–46.

35. Roux CM, Butler BP, Chau JY, et al. Both hemolytic anemia and malaria parasite-specific factors increase susceptibility to nontyphoidal *Salmonella enterica* serovar Typhimurium infection in mice. Infect Immun **2010**; 78:1520–7.

36. Lokken KL, Mooney JP, Butler BP, et al. Malaria parasite infection compromises control of concurrent systemic non-typhoidal *Salmonella* infection via IL-10-mediated alteration of myeloid cell function. PLoS Pathog **2014**; 10:e1004049.

37. Harchut K, Standley C, Dobson A, et al. Over-diagnosis of malaria by microscopy in the Kilombero Valley, southern Tanzania: an evaluation of the utility and cost-effectiveness of rapid diagnostic tests. Malar J **2013**; 12:159.

38. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M. The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev **2010**; 23:235–51.

39. Walsh AL, Phiri AJ, Graham SM, Molyneux EM, Molyneux ME. Bacteremia in febrile Malawian children: clinical and microbiologic features. Pediatr Infect Dis J **2000**; 19:312–8.

40. Lunguya O, Lejon V, Phoba MF, et al. Antimicrobial resistance in invasive nontyphoid *Salmonella* from the Democratic Republic of Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum data lactamases. PLoS Negl Trop Dis **2013**; 7:e2103.