Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study

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Summary

Background Available incidence data for invasive salmonella disease in sub-Saharan Africa are scarce. Standardised, multicountry data are required to better understand the nature and burden of disease in Africa. We aimed to measure the adjusted incidence estimates of typhoid fever and invasive non-typhoidal salmonella (iNTS) disease in sub-Saharan Africa, and the antimicrobial susceptibility profiles of the causative agents.

Methods We established a systematic, standardised surveillance of blood culture-based febrile illness in 13 African sentinel sites with previous reports of typhoid fever: Burkina Faso (two sites), Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar (two sites), Senegal, South Africa, Sudan, and Tanzania (two sites). We used census data and health-care records to define study catchment areas and populations. Eligible participants were either inpatients or outpatients who resided within the catchment area and presented with tympanic (≥38.0°C) or axillary temperature (≥37.5°C). Inpatients with a reported history of fever for 72 h or longer were excluded. We also implemented a health-care utilisation survey in a sample of households randomly selected from each study area to investigate health-seeking behaviour in cases of self-reported fever lasting less than 3 days. Typhoid fever and iNTS disease incidences were corrected for health-care-seeking behaviour and recruitment.

Findings Between March 1, 2010, and Jan 31, 2014, 135 Salmonella enterica serotype Typhi (S Typhi) and 94 iNTS isolates were cultured from the blood of 13 431 febrile patients. Salmonella spp accounted for 33% or more of all Salmonella enterica isolates in 27% (95% CI 24–30) of 27% (95% CI 24–30) of isolates from Ghana, Kenya, and Tanzania. Multidrug-resistant S Typhi was isolated in Ghana, Kenya, and Tanzania (both sites combined), and multidrug-resistant iNTS was isolated in Burkina Faso (both sites combined), Ghana, Kenya, and Guinea-Bissau.

Interpretation Typhoid fever and iNTS disease are major causes of invasive bacterial febrile illness in the sampled locations, most commonly affecting children in both low and high population density settings. The development of iNTS vaccines and the introduction of S Typhi conjugate vaccines should be considered for high-incidence settings, such as those identified in this study.

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Research in context

Evidence before this study

We did a literature search using PubMed with the following search terms: ("typhoid" OR "typhoid fever" OR "Salmonella Typhi" OR "S Typhi" OR "salmonella infection" OR "enteric fever" OR "non-typhoidal salmonella" OR "NTS") AND ("incidence" OR "rate" OR "frequency" OR "prevalence" OR "morbidity" OR "burden" OR "surveillance" OR "epidemiology"). We restricted publication dates from Dec 31, 1995, to July 30, 2016, and no language restrictions were applied. The date of our last search was July 30, 2016.

Salmonella infections are a major cause of global morbidity and mortality; however, substantial knowledge gaps exist with regards to the distribution and incidence of disease caused by Salmonella enterica serotype Typhi and invasive non-typhoidal salmonella (NTS) disease in sub-Saharan Africa.

Before the Typhoid Fever Surveillance in Africa Program (TSAP), estimates of typhoid fever incidence data from Africa were available from four vaccine trials and one population-based study in Kenya. Other estimates of invasive salmonella infections originated from different descriptions of bacteriaemia in febrile patients in The Gambia, Malawi, Mozambique, and Kenya. These few, unstandardised, published data are not sufficient for understanding the burden of the disease in sub-Saharan Africa.

In 2008, WHO expressed the necessity for more epidemiological information to estimate the incidence and antimicrobial susceptibility of invasive salmonella disease. Consequently, in January, 2009, the International Vaccine Institute (Seoul, South Korea) and the Kenya Medical Research Institute (Kilifi, Kenya) co-hosted a meeting with five other international consortium members to form a network of 13 surveillance sites across ten countries, and implemented cross-sectional studies to investigate the health-care-seeking behaviour of the populations under surveillance.

Added value of this study

Original data collected in TSAP represent the most comprehensive standardised analysis done in sub-Saharan Africa of the incidence and antimicrobial resistance patterns of invasive salmonella infections. The results describe the incidence estimated, adjusted by health-care-seeking behaviour, and antimicrobial susceptibility of typhoid fever and NTS diseases from 13 sites in ten sub-Saharan Africa countries. For typhoid fever disease, we estimate that the overall incidence is two to three times higher than five times that previously estimated.

Implications of all the available evidence

The results of this study underscore the need for preventive measures, including vaccines, improved sanitation and hygiene, malaria control, antiretroviral therapy programmes, and improved nutrition. The results also emphasise that the implementation of effective antimicrobials might be impaired by the presence and potential increase of drug-resistance salmonella strains in the region. The advent of typhoid conjugate vaccines might provide more powerful tools to control typhoid fever; the first vaccine, which was manufactured in India, has already been submitted to WHO for prequalification. Data from this study will be included in the GAVI Alliance review of potential subsidies for typhoid fever vaccines in 2017; their recommendation will be crucial for the deployment of these vaccines. Hence, an urgent need exists to understand the pragmatic aspects of vaccine targeting and delivery, particularly given the burden of disease in children, the associated risk factors, and the focal nature of the disease. Further assessment of the incidence in infants (0–5 months vs 6–11 months) and data on severe typhoid fever or iNTS, including mortality, is crucial to determine the potential effect of future vaccines. Our follow-on study—Severe Typhoid in Africa (SETA)—which investigates severe typhoid burden, is underway.

WHO, in 2008, to request more epidemiological information to reliably estimate the incidence of typhoid fever and iNTS disease and the antimicrobial susceptibilities of the corresponding organisms. Consequently, between 2010, and 2014, we established 13 surveillance sites across sub-Saharan Africa in...
locations where typhoid fever had been previously reported. This network formed the Typhoid Fever Surveillance in Africa Program (TSAP) and served as a platform to implement standardised surveillance of febrile illness and cross-sectional studies to investigate the health-care-seeking behaviour of the surveyed populations. Here, we present the adjusted incidence estimates of typhoid fever and iNTS disease and the antimicrobial susceptibility profiles of the causative agents at the 13 selected surveillance sites.

Methods
Study design, site selection, and participants
We used a multicentre, population-based, prospective surveillance study design. Selection of the surveillance sites in sub-Saharan Africa was not random; locations were eligible if they had evidence of previous typhoid fever, a laboratory infrastructure suitable for blood culture, an onsite health-care facility, and staff experienced in microbiological laboratory research. 13 sites in ten countries were selected (figure 1), four of which already had established surveillance systems: Pietermaritzburg, South Africa; Asante Akim North, Ghana; Moshi Urban District and Moshi Rural District, Tanzania; and Kibera, Kenya. Four sites were part of the International Network for the Demographic Evaluation of Populations and Their Health (INDEPTH): Polesgo and Nioko II, Burkina Faso; Butajira, Ethiopia; and Bandim, Guinea-Bissau. These sites had functional Health and Demographic Surveillance Systems (HDSS) in place. Additional surveillance sites were Isotry and Imerintsiatosika, Madagascar; Pikine, Senegal; and East Wad Medani, Sudan. The surveillance system in Kibera was established before TSAP with an active, population-based surveillance component. Home visits were done once every 2 weeks to screen for febrile patients and encourage visits to the affiliated health-care facility. Active surveillance in Kibera was continued throughout

Figure 1: Sites participating in the Typhoid Fever Surveillance in Africa Program
Surveillance site

| Country      | Site Health-care facility records | Patients with febrile illness from study area | Study database records | Total patients recruited | Total patients analysed |
|--------------|----------------------------------|---------------------------------------------|------------------------|-------------------------|-------------------------|
| Burkina Faso | Nioko II                          | 4204†                                        | NA‡                    | 918                     | 763                     |
| Kenya        | Pokcro                           | 2180†                                        | NA‡                    | 574                     | 425†                    |
| Madagascar   | Bongolava                        | 1364                                         | NA‡                    | 1251                    | 997                     |
| Ghana        | Asante Akim North                | 5822                                         | NA‡                    | 2651                    | 1021                    |
| Guinea-Bissau | Bandim                         | 5531                                         | NA‡                    | 2684                    | 1054                    |
| Sudan        | East Ward Mede                   | 574                                          | NA‡                    | 596                     | 328                     |
| Tanzania     | Moshi                                           | 14361                                        | NA‡                    | 847                     | 308‡                    |
| Ethiopia     | Kutale                           | 960                                          | NA‡                    | 847                     | 308‡                    |
| Senegal      | PKome                            | 1108                                         | NA‡                    | 1058                    | 378‡                    |
| South Africa | Pieternatta Borg                | 1260                                         | NA‡                    | 1128                    | 444                     |

Figure 2: Visits to health-care facilities and recruitment of patients during surveillance period at each site

NA=not available. *Data on health facility visits were collected retrospectively, after completion of surveillance period. Diagnosis of febrile illnesses was used at sites when temperature of patients was not recorded. †Number estimated by the proportion of the population under demographic surveillance at each respective site. ‡In Tanzania, before Nov 11, 2011, every fifth eligible patient was recruited; from Nov 11, 2011, every second eligible patient was recruited. This recruitment pattern was applied to this number.

For the study protocol see http://www.ivi.int/?page_id=12473&uid=922&mod=document

TSAP. All other sites implemented passive surveillance.10 The ethics committees of all collaborating institutions and the International Vaccine Institute (Seoul, South Korea) approved the study protocol. The catchment area for each site was determined through health-care facility records and through accessible administrative and demographic data.11 We determined the population of each catchment area using the latest census or the INDEPTH database. We categorised sites as urban, rural, or other using setting classifications at each site. Surveillance was implemented in each study location for a period of at least 12 months and recruitment occurred at primary, secondary, and tertiary health-care facilities.

Recruitment was open to outpatients and inpatients who visited any of the health-care facilities participating in TSAP, who resided within the catchment area and presented with typanic (≥38·0°C) or axillary temperature (≥37·5°C). Inpatients with a reported history of fever for 72 h or longer were excluded, as were patients with residence outside of the catchment area. Asante Akim North recruited children younger than age 15 years only; other sites recruited patients of all ages. Written informed consent preceded recruitment and clinical appraisal forms were completed for all participants.

Laboratory procedures

We standardised laboratory, quality control, and blood sample collection procedures across sites.19 Blood (5–10 mL for adults; 1–3 mL for children) was inoculated into aerobic blood culture bottles and incubated in an automated blood culture system (BD BACTEC, Becton-Dickinson, USA, or BacT/ALERT, BioMérieux, France), with the exception of Sudan, where manual culturing with daily subculturing for up to 5 days was instituted. Gram staining and bacterial identification were done with standard microbiological techniques.14 Quality control of preanalytical processes included time and temperature control measures, during which every blood culture bottle was collected, transported, and placed into the incubator. Quality control of analytical processes included sterility and function control of culture media, controls of biochemical reactions, and antimicrobial susceptibility testing. For the quality control of manual culturing in Sudan, additionally, blood culture bottles were inoculated weekly with a suspension containing *Escherichia coli* or *Staphylococcus aureus* references. Inoculated blood culture bottles were incubated overnight and verified for growth by subculture.

Contaminants were defined as organisms not typically associated with bloodstream infections; these included non-pathogens and those more commonly associated with commensal skin microbiota, including coagulase-negative *Staphylococci*, *Bacillus* spp, and *Micrococcus* spp. Antimicrobial susceptibility testing was done by disc diffusion according to Clinical and Laboratory Standards Institute17 standards for ampicillin, amoxicillin-clavulanic acid, chloramphenicol, co-trimoxazole, ceftriaxone, and ciprofloxacin. Multidrug resistance was defined as resistance to ampicillin or amoxicillin-clavulanic acid, chloramphenicol, and co-trimoxazole. Isolates with intermediate susceptibility were classified as resistant. Malaria blood smears were routinely done, except in South Africa. In Ethiopia, rapid diagnostic tests (SD BIOLINE Malaria Ag Pf/Pv, SD Standard Diagnostics, Yongin, South Korea) were used in addition to routine malaria blood smears.
### Surveillance sites

| Surveillance sites | Type of health facility (IPD, OPD) | Setting† | Population density, people per km² | Surveillance period (months)‡ | Source of catchment population | Collaborating research institution |
|-------------------|-----------------------------------|----------|-----------------------------------|-------------------------------|---------------------------------|-----------------------------------|
| Nioko II, Burkina Faso | 1 hospital (IPD, OPD) | Semi-urban | 22 044 | April, 2012, to September, 2013 (18) | Ministry of Health 2012 | UoO |
| Polesgo, Burkina Faso | 1 health-care centre (OPD) | Semi-urban | 5 163 | April, 2012, to September, 2013 (18) | HDSS 2011§ | UoO |
| Bandim, Guinea-Bissau | 1 hospital, 1 health-care centre (IPD, OPD) | Urban | 17 078 | December, 2011, to April, 2013 (17) | HDSS 2012§ | BHP |
| Pikine, Senegal | 1 hospital (IPD) | Urban and urban slum | 16 695 | March, 2010, to May, 2012 (27) | Census 2010|| | |
| Asante Akim North, Ghana | 1 hospital, 3 health-care centres (OPD) | Urban and rural | 121 | July, 2012, to January, 2013 (13) | Census 2008** | KCCR/BNITM |
| East Wad Medani, Sudan | 1 hospital (IPD) | Rural | 72 099 | May, 2012, to January, 2014 (21) | Census 2012| | |
| Butajira, Ethiopia | 1 hospital, 3 health-care centres (OPD) | Rural | 65 45 | November, 2011, to June, 2013 (20) | Census 2012| | |
| Imernitiato-sika, Madagascar | 1 health-care centre (OPD) | Semi-urban and rural | 225 | February, 2012, to May, 2013 (16) | Census 2010†† | KCCR/BNITM |
| Isotry, Madagascar | 1 hospital (IPD, OPD) | Urban | 29 301 | September, 2011, to May, 2013 (21) | Census 2012| | |
| Pietermaritzburg, South Africa | 1 hospital (IPD) | Urban | 1191 | February, 2012, to January, 2014 (24) | Census 2012|| | |
| Moshi Urban District, Tanzania | 1 hospital (IPD, OPD) | Urban | 3 069 | September, 2011, to May, 2013 (21) | Census 2012| | |
| Moshi Rural District, Tanzania | 1 hospital (IPD, OPD) | Rural | 3 32 | January, 2012, to December, 2013 (24) | KEMRI/CDC | KEMRI/CDC |
| Kibera, Kenya* | 1 health-care centre (OPD) | Urban slum | 77 000 | January, 2012, to December, 2013 (24) | KEMRI/CDC | KEMRI/CDC |

### Patient demographics

| Patient demographics | Patients analysed, N§§ | Median age, years (IQR) | 0-1 year, n (% of N) | 2-4 years, n (% of N) | ≥15 years, n (% of N) | Female patients, n (% of N) | Inpatients, n (% of N) |
|----------------------|------------------------|------------------------|----------------------|------------------------|------------------------|--------------------------|----------------------|
| Nioko II, Burkina Faso | 1198 | 4 (1–14) | 247 (27%) | 235 (26%) | 228 (25%) | 467 (51%) | 66 (7%) |
| Polesgo, Burkina Faso | 756 | 7 (3–21) | 117 (15%) | 148 (20%) | 252 (33%) | 404 (53%) | NA |
| Bandim, Guinea-Bissau | 1021 | 3 (1–7) | 114 (12%) | 272 (22%) | 274 (27%) | 487 (48%) | 224 (22%) |
| Pikine, Senegal | 1058 | 10 (0–5) | 9 (1%) | 23 (2%) | 255 (24%) | 486 (44%) | 241 (23%) |
| Asante Akim North, Ghana | 2651 | 15 (5–25) | 114 (42%) | 841 (32%) | 696 (26%) | 1204 (45%) | 2651 (100%) |
| East Wad Medani, Sudan | 644 | 20 (9–32) | 7 (4%) | 41 (6%) | 275 (43%) | 348 (54%) | 31 (4%) |
| Butajira, Ethiopia | 847 | 26 (17–40) | 7 (3%) | 46 (5%) | 303 (36%) | 433 (51%) | NA |
| Imernitiato-sika, Madagascar | 976 | 3 (1–29) | 7 (2%) | 12 (1%) | 319 (19%) | 570 (58%) | NA |
| Isotry, Madagascar | 1501 | 11 (2–39) | 11 (1%) | 12 (1%) | 209 (19%) | 597 (66%) | NA |
| Pietermaritzburg, South Africa | 1128 | 14 (2–39) | 11 (1%) | 14 (1%) | 206 (19%) | 586 (52%) | NA |
| Moshi Urban District, Tanzania | 406 | 19 (2–39) | 14 (1%) | 14 (1%) | 301 (24%) | 211 (52%) | NA |
| Moshi Rural District, Tanzania | 240 | 26 (9%) | 14 (1%) | 14 (1%) | 14 (1%) | 14 (1%) | NA |
| Kibera, Kenya* | 1251 | 7 (4–14) | 11 (1%) | 14 (1%) | 14 (1%) | 14 (1%) | NA |

Table 1 continues on next page
Health-care utilisation survey and person-years of observation calculation

The health-care-seeking behaviour of the populations under surveillance was investigated with the assumption that access to the TSAP health-care facility was non-uniform throughout the population. A standardised and pretested health-care utilisation survey was implemented in a representative sample of households randomly selected from each study area. We investigated health-care-seeking behaviour in cases of self-reported fever lasting less than 3 days. The first choice of health-care facility in cases of fever was categorised by age-stratified groups and used to calculate the proportion of individuals from the catchment population who visited this TSAP health-care facility. This proportion constituted an adjustment factor to correct incidences. The time at risk in person-years of observation (PYO) stratified by age was calculated using the adjusted population. In HDSS sites, each resident contributed to PYO for the time present in the study area during the recruitment period. In non-HDSS sites, we calculated PYO by projecting the catchment population from the start to the end of the study recruitment period, and multiplied the calculated average population by the number of years of surveillance duration.

Statistical analysis

We established a multicountry database using FoxPro software. We excluded patients from the analysis who were recruited during pilot testing, failed to meet inclusion criteria, or had incomplete laboratory results. We estimated incidences per 100 000 PYO. Confirmed invasive salmonella cases, stratified by age group (0–1 years, 2–4 years, 5–14 years, and ≥15 years), were adjusted by the specific age-group recruitment proportion. We calculated this proportion by dividing the number of patients with complete data (numerator) by the total number of patients in the study area who had been diagnosed with a febrile illness at a recruitment facility during the surveillance period (denominator). The catchment population in PYO, adjusted by the health-care-seeking behaviour in cases of self-reported fever lasting less than 3 days, was used as the denominator in crude and adjusted incidence rates (AIR). The 95% CI for AIR was derived on the log-scale and exponentiated. We used the error factor (exp[^1.96/√adjusted cases]) to calculate the lower (adjusted rate/error factor) and upper (adjusted rate × error factor) 95% CIs. At the sites in Senegal, Ethiopia, and South Africa, incomplete health-care facility records did not allow for the estimation of the recruitment proportion and calculation of AIRs; for these sites we present crude rates. AIRs for typhoid fever and iNTS were assessed for all other sites. Differences in proportions of blood cultures positive for a pathogen between study years were assessed with the χ² test (SAS, version 9.3).
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 1, 2010, and Jan 31, 2014, we recruited 13 558 patients from 13 sites who met the inclusion criteria and resided in the catchment areas (figures 1, 2). We excluded data from 127 (1%) patients because of incomplete laboratory results; data from 13 431 patients were analysed, and 8582 patients (64%) were younger than 15 years (table 1). All patients had one blood culture sample analysed at recruitment and 11 421 (85%) were screened for malaria parasites (table 1). The proportion of contaminated blood cultures ranged from less than 1% in Imerintsiatosika to 24% in Nioko II. The proportion of blood cultures that yielded non-contaminant bacteria varied between sites, ranging from 1% in Imerintsiatosika to 9% in Kibera (table 1). In total, 568 non-contaminant bacteria were isolated from blood samples of febrile patients. The most frequent non-contaminant bacteria isolated were S Typhii (135 [24%]), NTS (94 [17%]), S aureus (70 [12%]), E coli (47 [8%]), and Streptococcus pneumoniae (43 [8%]). Of the sites with at least 2 years of surveillance (Asante Akim North, Kibera, and Pietermaritzburg), the proportion of blood cultures that were pathogen positive differed significantly between study years in Kibera only (12% at year 1 and 5% at year 2; p<0·0001; χ² test).

With the exception of East Wad Medani, Salmonella spp were isolated from the blood of febrile patients at all sites (135 S Typhi and 94 iNTS isolates), which accounted for 33% or more of all isolated bacteria in all but four sites (East Wad Medani, Pietermaritzburg, Butajira, and Isotry). Seasonal variation was not observed at any site (data not shown). The most common iNTS serovars were S enterica serotype Typhimurium (38 [40%] of 94), S enterica serotype Enteridis (11 [12%] of 94), and S enterica serotype Dublin (10 [11%] of 94). The highest AIRs for typhoid fever in the 15 years or younger age group were observed in Polesgo, Kibera, and Asante Akim North (table 2). S Paratyphi A (three isolates) was isolated in Senegal only.

Among age groups of children younger than 15 years, the highest AIR for typhoid fever was observed in children aged 2–4 years from Polesgo, Asante Akim North, Moshi Urban District, and Kibera, and in children aged 5–14 years from Kibera and Polesgo (table 2). The AIR for typhoid fever in adults (aged ≥15 years) was less than 70 per 100 000 PYO at all sites except Moshi Urban District, Kibera, and Polesgo (table 2).

iNTS organisms were more frequently isolated from infants (0–1 years) or children aged 2–4 years than from adults (table 2), except for the sites in Pikine, Moshi Rural District, and Kibera. The AIR for iNTS among children aged 2–4 years was highest in Nioko II, Polesgo, and Asante Akim North. The AIR for iNTS in children younger than 15 years was less than 100 per 100 000 PYO in Kibera, Imerintsiatosika, and in both sites in Tanzania. No iNTS was isolated from sites in Sudan, South Africa, Ethiopia, and Isotry.

The antimicrobial susceptibility profiles of S Typhi and iNTS isolates differed between sites (table 3). Overall, 47% of S Typhi isolates and 48% of iNTS isolates were multidrug resistant. Most multidrug-resistant S Typhi isolates were obtained at the sites in Kenya, Ghana, and Tanzania (both sites combined). Multidrug-resistant iNTS isolates were isolated at the sites in Burkina Faso (both combined), Ghana, Guinea-Bissau, and Kenya (table 3). S Typhi isolates that had reduced ciprofl oxacin susceptibility were cultured in Kenya and South Africa, only; one ciprofl oxacin-resistant S Paratyphi A organism was isolated in Senegal. Ciprofl oxacin-resistant iNTS was similarly uncommon, isolated only in Burkino Faso (once at the Nioko II site) and in Ghana. One iNTS isolate in Kenya was resistant to ceftriaxone (table 3).

Discussion

This study identified Salmonella as a major cause of invasive bacterial febrile illness across sub-Saharan Africa, affecting children aged 2–14 years rather than adults, and arising in both high-population and low-population density settings. Other major causes of invasive bacterial febrile illnesses varied by country; E coli and S aureus were the most frequent non-Salmonella pathogens isolated from blood.

Results from previous studies suggest that typhoid fever in some sub-Saharan Africa settings occurs predominately in urban settlements with high-population densities, and that disease incidence could have been overestimated by the use of the Widal test. Our study, done using a standardised protocol in both urban and rural settings, indicated high incidences of typhoid fever and iNTS in areas with high-population and low-population densities. Separate analyses done at the Ghana site confirmed this observation and revealed a higher disease incidence in children living in rural areas than in those living in urban areas. Furthermore, we observed variable incidences of typhoid fever and iNTS among neighbouring populations in Burkina Faso, and in the same populations in Kenya and Ghana in consecutive years, indicating a focal nature and a fluctuating burden of iNTS disease.

A previous global estimate of the burden of typhoid fever indicated that south-central and east-central Asia had the highest incidences of typhoid fever with more than 100 cases per 100 000 people annually; Africa was estimated to have a medium incidence (10–100 cases per 100 000). The AIR for typhoid fever estimated in our
### Table 2: Proportion of individuals from study population visiting recruitment facility in case of fever (95% CI)

| Study | PYO estimation | Recruitment proportion | Study population | Study population adjusted by health-seeking behaviour |
|-------|----------------|------------------------|------------------|------------------------------------------------------|
|       | Crude cases    | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
|       | (95% CI)       | (95% CI)               | (95% CI)         | (95% CI)                                             |

#### Nioko II, Burkina Faso

- **0–1 years**: 81% (74–88) Crude cases 2208, Crude incidence per 100 000 PYO 1788, Cases adjusted for recruitment 2097, Adjusted incidence per 100 000 PYO (95% CI) 247/1297 (19%).
- **2–4 years**: 81% (75–86) Crude cases 1823, Crude incidence per 100 000 PYO 1477, Cases adjusted for recruitment 2097, Adjusted incidence per 100 000 PYO (95% CI) 251/1395 (19%).
- **5–14 years**: 81% (78–84) Crude cases 4295, Crude incidence per 100 000 PYO 3677, Cases adjusted for recruitment 4889, Adjusted incidence per 100 000 PYO (95% CI) 355/2161 (26%).
- **<15 years**: Na Crude cases 8326, Crude incidence per 100 000 PYO 7544, Cases adjusted for recruitment 9083, Adjusted incidence per 100 000 PYO (95% CI) 555/2757 (20%).
- **≥15 years**: 81% (79–83) Crude cases 9428, Crude incidence per 100 000 PYO 7637, Cases adjusted for recruitment 10676, Adjusted incidence per 100 000 PYO (95% CI) 208/759 (27%).

#### Polesgo, Burkina Faso

- **0–1 years**: 92% (86–99) Crude cases 896, Crude incidence per 100 000 PYO 729, Cases adjusted for recruitment 824, Adjusted incidence per 100 000 PYO (95% CI) 117/475 (25%).
- **2–4 years**: 83% (76–89) Crude cases 856, Crude incidence per 100 000 PYO 710, Cases adjusted for recruitment 992, Adjusted incidence per 100 000 PYO (95% CI) 148/466 (32%).
- **5–14 years**: 87% (83–91) Crude cases 1734, Crude incidence per 100 000 PYO 1509, Cases adjusted for recruitment 2104, Adjusted incidence per 100 000 PYO (95% CI) 255/510 (49%).
- **<15 years**: Na Crude cases 3486, Crude incidence per 100 000 PYO 3043, Cases adjusted for recruitment 3860, Adjusted incidence per 100 000 PYO (95% CI) 277/1259 (26%).
- **≥15 years**: 87% (84–89) Crude cases 4088, Crude incidence per 100 000 PYO 3557, Cases adjusted for recruitment 4957, Adjusted incidence per 100 000 PYO (95% CI) 34/16- (27%).

#### Bandim, Guinea-Bissau

- **0–1 years**: 46% (39–54) Crude cases 10852, Crude incidence per 100 000 PYO 4992, Cases adjusted for recruitment 5198, Adjusted incidence per 100 000 PYO (95% CI) 206/631 (33%).
- **2–4 years**: 43% (37–48) Crude cases 7307, Crude incidence per 100 000 PYO 710, Cases adjusted for recruitment 3142, Adjusted incidence per 100 000 PYO (95% CI) 175/359 (49%).
- **5–14 years**: 42% (41–48) Crude cases 19905, Crude incidence per 100 000 PYO 1886, Cases adjusted for recruitment 11101, Adjusted incidence per 100 000 PYO (95% CI) 815/1780 (49%).
- **<15 years**: Na Crude cases 3964, Crude incidence per 100 000 PYO 1649, Cases adjusted for recruitment 20165, Adjusted incidence per 100 000 PYO (95% CI) 10/1 (49%).
- **≥15 years**: 45% (43–47) Crude cases 62694, Crude incidence per 100 000 PYO 2312, Cases adjusted for recruitment 7109, Adjusted incidence per 100 000 PYO (95% CI) 10/1 (49%).

| Study | PYO estimation | Recruitment proportion | Study population | Study population adjusted by health-seeking behaviour |
|-------|----------------|------------------------|------------------|------------------------------------------------------|
|       | Crude cases    | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
|       | (95% CI)       | (95% CI)               | (95% CI)         | (95% CI)                                             |

#### Table 2 continues on next page
| Asante Akim North, Ghana | Study population adjusted by health-seeking behaviour | PYO estimation | Recruitment proportion | Salmonella Typhi | INTS |
|--------------------------|--------------------------------------------------|----------------|------------------------|----------------|------|
|                          | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
| 0–1 years                | 16% (14–18) | 11 222 | 1 760 | 4 080 | 41%* | 2 | 49 | 49 | 120 (49–290) | 29 | 71 | 71 | 172 (137–218) |
| 2–4 years                | 16% (13–18) | 8 086 | 1 718 | 2 940 | 41%* | 13 | 442 | 317 | 1079 (762–1528) | 23 | 782 | 56 | 1908 (1469–2479) |
| 5–14 years               | 16% (15–17) | 34 439 | 5 415 | 12 554 | 62/1657 (38%) | 15 | 119 | 395 | 314 (230–430) | 7 | 56 | 18 | 147 (93–232) |
| <15 years                | NA | 5 374 | 8 444 | 19 374 | NA | 30 | 153 | 76 | 389 (310–486) | 59 | 291 | 145 | 742 (631–873) |
| ≥15 years                | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| All                      | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Pikine, Senegal‡         | Study population adjusted by health-seeking behaviour | PYO estimation | Recruitment proportion | Salmonella Typhi | INTS |
|                          | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
| 0–1 years                | 39% (32–46) | 20 120 | 7 837 | 1 104 | NA | 0 | 0 | NA | NA | 0 | 0 | NA | NA |
| 2–4 years                | 37% (33–41) | 3 018 | 1 109 | 1 585 | NA | 0 | 0 | NA | NA | 0 | 0 | NA | NA |
| 5–14 years               | 31% (28–34) | 96 152 | 2 907 | 4 577 | NA | 3 | 7 | NA | NA | 1 | 7 | NA | NA |
| <15 years                | NA | 146 452 | 4 841 | 6 623 | NA | 3 | 4 | NA | NA | 0 | 0 | NA | NA |
| ≥15 years                | 30% (28–31) | 195 726 | 5 818 | 8 347 | NA | 4 | 5 | NA | NA | 3 | 6 | NA | NA |
| All                      | NA | 342 178 | 10 459 | 13 496 | NA | 7 | 5 | NA | NA | 4 | 5 | NA | NA |
| East Wad Medani, Sudan§  | Study population adjusted by health-seeking behaviour | PYO estimation | Recruitment proportion | Salmonella Typhi | INTS |
|                          | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
| 0–1 years                | 23% (14–32) | 2 377 | 527 | 589 | 2/85(2%) | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| 2–4 years                | 22% (15–29) | 3 566 | 781 | 857 | 29/108 (27%) | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| 5–14 years               | 25% (21–28) | 11 071 | 2 735 | 2 999 | 160/234 (68%) | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| <15 years                | NA | 17 014 | 4 053 | 4 445 | NA | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| ≥15 years                | 29% (27–31) | 29 843 | 8 684 | 9 525 | 131/147 (89%) | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| All                      | NA | 46 857 | 12 737 | 13 970 | NA | 0 | 0 | 0 | 0 (0–0) | 0 | 0 | 0 | 0 (0–0) |
| Butajira, Ethiopia§      | Study population adjusted by health-seeking behaviour | PYO estimation | Recruitment proportion | Salmonella Typhi | INTS |
|                          | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
| 0–1 years                | 69% (59–78) | 2 266 | 1 756 | 2 798 | NA | 0 | 0 | NA | NA | 0 | 0 | NA | NA |
| 2–4 years                | 62% (55–69) | 3 398 | 2 107 | 3 771 | NA | 0 | 0 | NA | NA | 0 | 0 | NA | NA |
| 5–14 years               | 65% (61–69) | 14 015 | 9 110 | 16 305 | NA | 1 | 6 | NA | NA | 0 | 0 | NA | NA |
| <15 years                | NA | 19 679 | 12 780 | 22 874 | NA | 1 | 4 | NA | NA | 0 | 0 | NA | NA |
| ≥15 years                | 65% (62–68) | 42 545 | 28 080 | 50 257 | NA | 2 | 4 | NA | NA | 0 | 0 | NA | NA |
| All                      | NA | 62 224 | 40 860 | 73 131 | NA | 3 | 4 | NA | NA | 0 | 0 | NA | NA |

(Continued from previous page)
| Study population adjusted by health-seeking behaviour | PYO estimation | Recruitment proportion | Salmonella Typhi iNTS | INTS |
|-----------------------------------------------------|----------------|------------------------|----------------------|------|
| Study population                                   | Code cases     | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Code cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
| Moshi Rural District, Tanzania                      |                |                        |                      |      |                |                        |                      |      |
| 0–1 years                                           | 4% (0–11)¶     | 24 289                 | 390                  | 693   | 79%*          | 0                      | 0                    | 0                   | 0 (0–0) |
| 2–4 years                                           | 2% (0–4)¶| | 25 281 | 406 | 721 | 79%* | 2 (4)** | 15 | 5 | 18 (8–44) | 0 | 0 | 0 (0–0) |
| 5–14 years                                          | 13% (10–16)    | 110 219               | 15 487               | 27 058 | 79%* | 2 (4)** | 14 | 5 | 18 (7–42) | 0 | 0 | 0 (0–0) |
| ≥15 years                                           | NA             | 167 789               | 16 283               | 28 922 | NA | 2 (4)** | 14 | 5 | 18 (7–42) | 0 | 0 | 0 (0–0) |
| ≥15 years                                           | 2% (1–2)      | 298 948               | 51 727               | 91 86 | 79%* | 1 (2)** | 22 | 2 | 28 (8–95) | 1 (2)** | 22 | 2.5 | 28 (8–95) |
| All                                                 | NA             | 466 737               | 21 454               | 38 108 | NA | 3 (6)** | 16 | 7 | 20 (10–41) | 1 (2)** | 5 | 2.5 | 7 (2–23) |
| Moshi Urban District, Tanzania                      |                |                        |                      |      |                |                        |                      |      |
| 0–1 years                                           | 7% (0–19)¶     | 10 406                 | 335                  | 595   | 79%*          | 0                      | 0                    | 0                   | 0 (0–0) |
| 2–4 years                                           | 2% (0–6)¶| | 10 831 | 348 | 618 | 79%* | 1 (5)** | 809 | 6.4 | 1028 (472–2257) | 0 | 0 | 0 (0–0) |
| 5–14 years                                          | 13% (8–18)     | 37 090                | 48 50                | 86 15 | 79%* | 2 (7)** | 81 | 8.9 | 103 (54–199) | 0 | 0 | 0 (0–0) |
| ≥15 years                                           | NA             | 58 546               | 55 33                | 98 28 | NA | 3 (12)** | 122 | 15.2 | 155 (94–256) | 1 (2)** | 20 | 2.5 | 26 (8–88) |
| ≥15 years                                           | 2% (0–3)      | 125 746               | 21 38                | 37 96 | 79%* | 3 (6)** | 158 | 7.6 | 201 (99–408) | 0 | 0 | 0 (0–0) |
| All                                                 | NA             | 184 292               | 76 71                | 136 26 | NA | 6 (18)** | 132 | 22.9 | 168 (111–253) | 1 (2)** | 15 | 2.5 | 19 (5–64) |
| Kibera, Kenya††                                     |                |                        |                      |      |                |                        |                      |      |
| 0–1 years                                           | 42% (38–47)    | 3467                   | 1456                 | 2031  | 99/99 (100%) | 3                    | 148 | 3 | 148 (48–458) | 1 | 49 | 1.0 | 49 (7–350) |
| 2–4 years                                           | 39% (30–42)    | 3070                   | 1197                 | 2039  | 312/312 (100%) | 10                | 490 | 10.0 | 490 (264–912) | 1 | 49 | 1.0 | 49 (7–350) |
| 5–14 years                                          | 43% (35–46)    | 3754                   | 3331                 | 5722  | 529/529 (100%) | 28               | 489 | 28.0 | 489 (338–709) | 1 | 17 | 1.0 | 17 (2–124) |
| ≥15 years                                           | NA             | 140 051               | 5884                 | 9792  | NA | 41 (12)** | 419 | 41.0 | 419 (308–569) | 3 | 31 | 3.0 | 31 (10–95) |
| ≥15 years                                           | 35% (32–38)    | 152 63                 | 5342                 | 9228  | NA | 13 (12)** | 141 | 13.0 | 141 (82–243) | 3 | 33 | 3.0 | 33 (10–101) |
| All                                                 | NA             | 184 292               | 76 71                | 136 26 | NA | 6 (18)** | 132 | 22.9 | 168 (111–253) | 1 (2)** | 15 | 2.5 | 19 (5–64) |
| Imerintsiatosika, Madagascar                       |                |                        |                      |      |                |                        |                      |      |
| 0–1 years                                           | 28% (20–37)    | 3424                   | 753                  | 1287  | 66/85 (78%) 5136 | 1130 | 1932 | 8/101 (86%) | 0 | 0 | 0 (0–0) |
| 2–4 years                                           | 19% (14–25)    | 5136                   | 1130                 | 1932  | 8/101 (86%) 0 | 0 | 0 | 0 (0–0) |
| 5–14 years                                          | 18% (15–20)    | 31 188                | 23 74                 | 40 57 | 18 456 (72%) 5 | 123 | 6.9 | 171 (81–360) | 0 | 0 | 0 (0–0) |
| ≥15 years                                           | NA             | 21 748               | 42 57                 | 7276  | NA | 5 | 69 | 6.9 | 95 (45–201) | 1 | 14 | 1.3 | 18 (3–99) |
| ≥15 years                                           | 17% (15–19)    | 24 652               | 41 87                 | 7153  | NA | 1 | 14 | 1.4 | 20 (4–103) | 0 | 0 | 0 (0–0) |
| All                                                 | NA             | 46 380               | 8444                 | 14 429 | NA | 6 | 42 | 8.4 | 58 (29–114) | 1 | 7 | 1.3 | 9 (2–50) |

(Table 2 continues on next page)
Table 2: Invasive salmonella infections across sites in the Typhoid Fever Surveillance in Africa Program

| Site                      | Study population adjusted by health-seeking behaviour | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) | Crude cases | Crude incidence per 100 000 PYO | Cases adjusted for recruitment | Adjusted incidence per 100 000 PYO (95% CI) |
|---------------------------|------------------------------------------------------|-------------|---------------------------------|---------------------------------|------------------------------------------|-------------|---------------------------------|---------------------------------|------------------------------------------|
| **Isotry, Madagascar**    |                                                      |             |                                 |                                 |                                          |             |                                 |                                 |                                          |
| 0–1 years                 | 6% (1–12)                                            | 3204        | 192                             | 261                             | 12/14 (86%)                             | 0           | 0                               | 0                               | 0 (0–0)                                  |
| 2–4 years                 | 10% (5–14)                                           | 4805        | 481                             | 653                             | 58/65 (89%)                             | 0           | 0                               | 0                               | 0 (0–0)                                  |
| 5–14 years                | 9% (7–11)                                            | 16386       | 14/75                           | 2005                            | 2342/88 (81%)                           | 1           | 50                               | 12                              | 62 (11–359)                              |
| <15 years                 | NA                                                   | 24395       | 214                             | 2919                            | NA                                       | 1           | 34                               | 12                              | 42 (7–247)                              |
| ≥15 years                 | 9% (7–11)                                            | 45328       | 413                             | 5621                            | 1137/1421 (81%)                         | 2           | 36                               | 24                              | 42 (12–151)                             |
| All                       | NA                                                   | 70323       | 628                             | 8540                            | NA                                       | 3           | 35                               | 36                              | 42 (15–119)                             |
| **Pietermaritzburg, South Africa** |                                                      |             |                                 |                                 |                                          |             |                                 |                                 |                                          |
| 0–1 years                 | 11% (5–17)                                           | 13990       | 151                             | 3055                            | NA                                       | 0           | 0                               | NA                              | NA                                       |
| 2–4 years                 | 7% (3–12)                                            | 20985       | 1490                           | 3013                            | NA                                       | 0           | 0                               | NA                              | NA                                       |
| 5–14 years                | 16% (13–19)                                          | 62313       | 1015                           | 20537                           | NA                                       | 0           | 0                               | NA                              | NA                                       |
| <15 years                 | NA                                                   | 97288       | 1315                           | 26605                           | NA                                       | 0           | 0                               | NA                              | NA                                       |
| ≥15 years                 | 15% (13–17)                                          | 294542      | 438                            | 88759                           | NA                                       | 2           | 2                               | NA                              | NA                                       |
| All                       | NA                                                   | 391830      | 5745                           | 115344                          | NA                                       | 2           | 2                               | NA                              | NA                                       |

Study population was adjusted for health-seeking behaviour and crude cases were adjusted for recruitment proportion (number of patients analysed divided by number of patients with febrile illness from study area who visited a recruitment health facility, multiplied by 100). iNTS=invasive non-typhoidal salmonella. NA=not available. PYO=person-years of observation. *Recruitment portion was not available for each age strata. †Target population for surveillance activities in Ghana included patients younger than 15 years of age; patients aged 15 years or older were not recruited. ‡Three Salmonella Paratyphi A were identified at this site, but are not included in this table. §No salmonella was isolated in Sudan. Missing data on recruitment patterns in Senegal, Ethiopia, and South Africa did not allow calculation of adjusted incidences. Crude rates are presented. ¶This proportion applies to age group 0–3 years, and it was used to adjust the study population by health-seeking behaviour. The adjusted populations in age groups 0–1 years and 1–4 years were added to estimate the total adjusted population age group 0–4 years. Subsequently, the percentage of children <2 years reported by the 2012 national census was applied to derive age groups 0–1 years and 2–4 years. **Crude cases have been adjusted for recruitment pattern unique to the site in Tanzania. Before Nov 11, 2011, every fifth eligible patient was recruited; from Nov 11, 2011, every second eligible patient was recruited. Adjusted cases (presented inside parentheses) were used to calculate crude rate. ††Active population mobilisation was done, in addition to passive surveillance.
study reveals a higher burden than previously estimated. Four sites had an overall AIR for typhoid fever of more than 100 per 100 000 PYO, five sites had an AIR for typhoid fever of more than 100 per 100 000 PYO in children younger than 15 years, and six sites had an AIR for typhoid fever of more than 100 per 100 000 PYO in at least one age group. Similar to the Diseases of the Most Impoverished programme done in Asia,21 our results show that children aged 2–14 years bear the greatest burden of typhoid fever. Notably, our data indicate that the AIR for typhoid fever at TSAP sites was equal to or even greater than incidences reported in five Asian countries in the early 2000s.29–32

For iNTS disease, we observed an AIR equal or higher than previously estimated and a bimodal age distribution with very young children and adults being the key age group for symptomatic infection.2 This age distribution differed from that observed for typhoid fever, in which children aged 2–14 years were the most affected, and emphasises substantial differences in the epidemiology of typhoid fever and iNTS disease. Malaria, malnutrition, and HIV infections have been reported to be associated with iNTS disease in Africa.21 At TSAP sites, a higher AIR for iNTS was observed in children with a malaria positivity rate of 30% or more than in those with a lower positivity rate; this observation was confirmed in a separate analysis.29

Results of our study identified a high prevalence of resistance against first-line antimicrobials in both S Typhi and iNTS infections. Reduced susceptibility to ciprofloxacin was identified in S Typhi from Kibera and Pietermaritzburg. Multidrug-resistant iNTS isolates were isolated at several sites and have been isolated in sub-Saharan Africa previously.25,26 Furthermore, a single iNTS isolate from Kibera showed resistance to ceftriaxone. Genomic analyses have described the spread of S Typhi haplotype H58 into Africa, a multidrug-resistant strain associated with reduced ciprofloxacin susceptibility. The susceptibility patterns observed in our study are concerning, particularly because some antimicrobial-resistant S Typhi can have a selective fitness advantage.24 Concerted measures are needed to monitor the emergence of fluoroquinolone-resistant Salmonella.

We made all efforts to minimise bias; however, our study has some limitations. First, we did not adjust the disease incidences for blood culture sensitivity, which is approximately 40–60% of bone marrow culture.33–37 This correction factor is inconsistently applied in studies and, if applied here, the incidences presented would double. The restricted sensitivity of blood culture to detect Salmonella pathogens applies to other bacterial pathogens as well—ie, S pneumoniae and Haemophilus influenzae type b—however, those are universally recognised as important infections for which vaccines are cost-effective, and vaccination programmes have been established. Second, our results represent incidence in sites selected because of their previous reports on typhoid fever. The site selection strategy limits the generalisability of the AIR to other locations and might result in the reduced detection of iNTS isolates.

### Table 3: Antimicrobial resistance patterns of Salmonella enterica serotype Typhi and iNTS isolates across sites

| Country         | S Typhi isolates, N | Isolate with antimicrobial resistance, n (%)† | Isolate with antimicrobial resistance, n (%)‡ |
|-----------------|---------------------|---------------------------------------------|---------------------------------------------|
|                 |                     | Ampicillin                                  | Amoxicillin-clavulanic acid                  |
| Burkina Faso    | 18                  | 10 (71%)                                    | 0                                           |
| Guinea-Bissau   | 3                   | 3 (21%)                                     | 0                                           |
| Senegal*        | 7                   | 12 (86%)                                    | 3                                           |
| Ghana           | 30                  | 13 (93%)                                    | 13 (93%)                                    |
| Ethiopia        | 3                   | 3 (21%)                                     | 3 (21%)                                     |
| Madagascar      | 9                   | 6 (67%)                                     | 3 (21%)                                     |
| South Africa    | 2                   | 2 (11%)                                     | 2 (11%)                                     |
| Tanzania        | 9                   | 9 (15%)                                     | 9 (15%)                                     |
| Kenya           | 54                  | 3 (6%)                                      | 3 (6%)                                      |
| All             | 135                 | 3 (3%)                                      | 3 (3%)                                      |

Resistant isolates are reported per country, rather than per site. No Salmonella enterica serotype Typhi (S Typhi) or iNTS isolates were cultured in Sudan. iNTS=invasive non-typhoidal salmonella. NR=no resistant isolates identified. †Seven S Typhi, four iNTS, and three S enterica serotype Paratyphi (S Paratyphi) isolates. One of the S Paratyphi isolates was resistant to ciprofloxacin. ‡Includes isolates fully and intermediately resistant against the respective drug, as defined by the Clinical Laboratory and Standards Institute guidelines 2013.21,22 (Defined as resistance against ampicillin or amoxicillin AND chloramphenicol AND co-trimoxazole.)
and the focal and unpredictable nature of the disease. Third, given the vast number of patients (and restricted diagnostics capacity), not every patient with a history of fever was enrolled—eg, at sites where inpatients were recruited, patients with a fever for 72 h or longer were excluded to minimise the inclusion of patients pretreated with antimicrobials and to maximise blood culture yield. Fourth, the proportion of the catchment population using the TSAP health-care facilities for febrile illness was low in some sites, and antimicrobial treatment before blood collection and its potential effect on blood culture sensitivity were not assessed. Fifth, the classification of the settings as either urban, rural, semi-urban, or urban-slum reflects the classification commonly used at each site and does not refer to a standard definition; instead, the population density of each site is presented to make setting comparisons. Sixth, sites with no previous experience of blood collection for blood culture assessment had a higher incidence of contamination than sites with previous experience of blood collection (South Africa, Ghana, Tanzania, and Kenya); these incidences might have led to errors in clinical interpretation and uncertainty to distinguish between clinically significant bacteraemia and contamination. Available isolates and blood samples collected from participants were PCR tested at the reference lab to minimise misclassification of isolated organisms. Seventh, the site in Ghana recruited only children younger than 15 years and the proportion of recruited inpatients varied greatly across all sites. Finally, data on disease severity, complications, mortality, and HIV status were not assessed because these were not primary study objectives. Despite these limitations, this multisite study, the largest study of typhoid fever and iNTS done across sub-Saharan Africa to date, provides the most current and accurate incidence figures for these major infectious diseases across the continent and has substantial implications for their control.

We surmise that the incidence of invasive salmonella infections among children in sub-Saharan Africa is much higher than previously estimated, underscoring the need for preventive measures. Therefore, until access to safe drinking water and improved sanitation is greatly expanded, the prevention of typhoid fever will require immunisation and effective treatment options.32 The advent of new typhoid fever conjugate vaccines might provide more powerful tools for disease control; the first typhoid fever conjugate vaccine (Bharat Biotech, Hyderabad) has been submitted to WHO for prequalification. Data from TSAP will be incorporated into the GAVI Alliances’ review of potential subsidies for typhoid fever vaccines in 2017; their recommendation will be crucial for deployment of these vaccines. Hence, the need to understand the pragmatic aspects of vaccine targeting and delivery is pressing, particularly given the burden of disease in children, the associated risk factors, and the focal and unpredictable nature of the disease. Similarly, in the absence of vaccines targeting iNTS disease, prevention will require a major investment in infrastructure for diagnosis and effective treatment of iNTS disease. When appropriate diagnosis and treatment are available, the use of effective antimicrobials might be impaired by the presence and potential increase of multidrug-resistant salmonella. Further assessment of incidences in infants (0–5 months vs 6–11 months) and data on severe typhoid fever or iNTS, including mortality, is crucial to determine the potential effect of future vaccines. We are currently undertaking a follow-on study—Severe Typhoid in Africa (SETA)—which investigates severe typhoid burden.

We conclude that typhoid fever and iNTS disease are major agents of invasive bloodstream infections in urban and rural locations, affecting children more commonly than adults across sub-Saharan Africa. Immunisation of high-risk age groups with existing and new vaccines should be a priority. The next generation of epidemiological studies in sub-Saharan Africa needs to provide better data regarding the severity and mortality of typhoid fever and iNTS to guide the introduction of new typhoid and iNTS vaccines. Lastly, the accelerated development and introduction of iNTS vaccines needs to become a fundamental goal on the global health agenda.

Contributors

FM and TFW contributed to study conception and design, analysis of data, interpretation of results, and drafting and editing of the paper. FK, JM, UP, VvK, EDM, and JDC contributed to study conception and design, data interpretation, and editing of the paper. MA, GDP, LMCE, VvK, and JKF contributed to data analysis. KT and BL contributed to study conception and design, data acquisition in the field, interpretation of the results, and editing of the paper. VvK, LMCE, SEP, CGM, CN, and JI drafted the manuscript and contributed to interpretation of results and editing of the paper. RFB, MA, FK, JM, UP, TFW, VvK, PA, YA-S, AA, MB-A, JAC, LMCE, JPD, NG, JTH, JJ, HJJ, KHK, JMM, RR, AGS, SEP, HJS, AS, MT, MBW, BY, MAET, HMB, LC, AJ, SVL, TMR, NS, and AT contributed to data acquisition in the field, interpretation of results, and editing of the paper. SB, JIC, UP, DMD, BSF, LPK, AAN, NVMH, BO, HR, TJLR, ES, HS-G, and AS contributed to laboratory work, interpretation of results, and editing of the paper. All authors read and approved the final draft.

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Declaration of interests

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References

1 Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ 2004; 82: 146–53.
2 Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. Global burden of invasive non-typhoidal Salmonella disease. Emerg Infect Dis 2013; 21: e941–49.
3 Langridge GC, Nair S, Wain J. Nontyphoidal Salmonella serovars cause different degrees of invasive disease globally. J Infect Dis 2009; 199: 602–03.
4 Mogasale V, Maskery B, Ochiai RL, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Global Health 2014; 2: e570–80.
5 Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. J Global Health 2012; 2: 020401.
6 Nielsen, MV, Sarpong, N, Krumkamp, R et al. Incidence and characteristics of bacteremia among children in rural Ghana. PLoS One 2012; 7: e44063.
7 Meek E, English M. Typhoid fever in children in Africa. Trop Med Int Health 2008; 13: 532–40.
8 Ochiai RL, Acosta CJ, Danovaro-Holliday MC, et al. A study of typhoid fever in five asian countries: Disease burden and implications for controls. Bull World Health Organ 2008; 86: 260–68.
9 Owall A, Sultana S, Zaman U, Riwer A, Zaidi AK. Incidence of typhoid bacteremia in infants and young children in southern coastal Pakistan. Pediatr Infect Dis J 2010; 29: 1035–39.
10 Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. Lancet 2012; 379: 2489–99.
11 Park SE, Pak GD, Aaby P, et al. The relationship between invasive non-Typhoidal Salmonella disease, other bloodstream infections, and malaria in sub-Saharan Africa. Clin Infect Dis 2016; 62: S23.
12 Takem EW, Roa A, Cunningham A. The association between malaria and non-typhoid Salmonella bacteremia in children in sub-Saharan Africa: a literature review. Malar J 2014; 13: 400.
13 Kaur J. Increasing antimicrobial resistance and narrowing therapeutics in Typhoidal Salmonellae. J Clin Diag Res 2013; 7: 576–79.
14 Vlieghe ER, Pla-Th, De Smet B, et al. Azithromycin and ciprofloxacin resistance in Salmonella bloodstream infections in cambodian adults. PLoS Negl Trop Dis 2012; 6: e1933.
15 Parry CM, Wijedoru L, Arjyal A, Baker S. The utility of diagnostic tests for enteric fever in endemic locations. Expert Rev Antimicrob Ther 2011; 9: 711–15.
16 Wain J, Hosoglu S, et al. The laboratory diagnosis of enteric fever. J Infect Dev Ctries 2008; 2: 421–25.
17 Baker S, Sarwar Y, Aiziz H, et al. Detection of Vi-negative Salmonella enterica serovar Typhi in the peripheral blood of patients with typhoid fever in the Faisalabad region of Pakistan. J Clin Microbiol 2005; 43: 4418–25.
18 Verma R, Baurwa M, Chawla S, Prinja S, Rajput M. New generation typhoid vaccines: an effective preventive strategy to control typhoid fever in developing countries. Hum Vaccin 2011; 7: 883–85.