Mtove, George; Amos, Ben; von Seidlein, Lorenz; Hendriksen, Ilse; Mwambuli, Abraham; Kimera, Juma; Mallahiyo, Rajabu; Kim, Deok Ryun; Ochiai, R Leon; Clemens, John D; +3 more... Reyburn, Hugh; Magesa, Stephen; Deen, Jacqueline L; (2010) Invasive salmonellosis among children admitted to a rural Tanzanian hospital and a comparison with previous studies. PloS one, 5 (2). e9244-. ISSN 1932-6203 DOI: https://doi.org/10.1371/journal.pone.0009244

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/4032/

DOI: https://doi.org/10.1371/journal.pone.0009244

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Invasive Salmonellosis among Children Admitted to a Rural Tanzanian Hospital and a Comparison with Previous Studies

George Mtove1,2, Ben Amos2,3, Lorenz von Seidlein2,4, Ilse Hendriksen2,5, Abraham Mwambuli2, Juma Kimera2,3, Rajabu Mallahiyo3, Deok Ryun Kim4, R. Leon Ochiai4, John D. Clemens4, Hugh Reyburn2,6, Stephen Magesa1,2, Jacqueline L. Deen2,4

1 National Institute for Medical Research - Amani Centre, Tanga, Tanzania, 2 Joint Malaria Programme, Moshi, Tanzania, 3 Teule Hospital, Muheza, Tanga, Tanzania, 4 International Vaccine Institute, Seoul, Korea, 5 Mahidol Oxford Research Unit, Bangkok, Thailand, 6 London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Background: The importance of invasive salmonellosis in African children is well recognized but there is inadequate information on these infections. We conducted a fever surveillance study in a Tanzanian rural hospital to estimate the case fraction of invasive salmonellosis among pediatric admissions, examine associations with common co-morbidities and describe its clinical features. We compared our main findings with those from previous studies among children in sub-Saharan Africa.

Methodology/Principal Findings: From 1 March 2008 to 28 Feb 2009, 1,502 children were enrolled into the study. We collected clinical information and blood for point of care tests, culture, and diagnosis of malaria and HIV. We analyzed the clinical features on admission and outcome by laboratory-confirmed diagnosis. Pathogenic bacteria were isolated from the blood of 156 (10%) children, of which 14 (9%) were S. typhi, 45 (29%) were NTS and 97 (62%) were other pathogenic bacteria. Invasive salmonellosis accounted for 59/156 (38%) bacteremic children. Children with typhoid fever were significantly older and presented with a longer duration of fever. NTS infections were significantly associated with prior antimalarial treatment, malarial complications and with a high risk for death.

Conclusions/Significance: Invasive salmonellosis, particularly NTS infection, is an important cause of febrile disease among hospitalized children in our rural Tanzanian setting. Previous studies showed considerable variation in the case fraction of S. typhi and NTS infections. Certain suggestive clinical features (such as older age and long duration of fever for typhoid whereas concomitant malaria, anemia, jaundice and hypoglycemia for NTS infection) may be used to distinguish invasive salmonellosis from other severe febrile illness.

Citation: Mtove G, Amos B, von Seidlein L, Hendriksen I, Mwambuli A, et al. (2010) Invasive Salmonellosis among Children Admitted to a Rural Tanzanian Hospital and a Comparison with Previous Studies. PLoS ONE 5(2): e9244. doi:10.1371/journal.pone.0009244

Editor: David Joseph Diemert, Sabin Vaccine Institute, United States of America

Received: December 2, 2009; Accepted: January 27, 2010; Published: February 16, 2010

Copyright: © 2010 Mtove et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was funded by a grant from the Korean International Cooperation Agency through the International Vaccine Institute (http://www.ivi.org/). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mtoveg2002@yahoo.co.uk

Introduction

Salmonella enterica serotype Typhi (S. typhi) and several non-Typhi serotypes of S. Enterica (NTS) are important causes of childhood bacteremia in African children [1]. Although the data is sparse, there seems to be a relatively low burden of pediatric typhoid fever across sub-Saharan Africa [2] whereas NTS has consistently been reported as a leading cause of bacteremia in African children [3,4]. In contrast to industrialized countries where NTS usually consists a self-limited gastroenteritis, invasive disease frequently occurs in sub-Saharan Africa with high case fatality rates among hospitalized children [1,3,4].

There is inadequate information on invasive salmonellosis in sub-Saharan Africa. In particular, the importance of NTS sepsis is not widely recognized. An important reason is that most hospitals in the region lack adequate microbiological facilities. Epidemiologic data on invasive Salmonellosis in sub-Saharan African countries is important for assisting clinical management and development of preventive strategies. We conducted fever surveillance in a district hospital in rural Tanzania. We estimated the case fraction of invasive salmonellosis among our pediatric admissions, examined associations with common co-morbidities and described its clinical features in comparison with other febrile illnesses. We compared our main findings with those from previous studies among children in sub-Saharan Africa.

Methods

Study Site and Population

The study was conducted at Teule Hospital, which is the designated district hospital of Muheza in north-eastern Tanzania. The hospital serves a catchment population of about 277,000 of
whom 17% are aged less than five years. Child mortality in the area is 165/1000 [3]. The majority of inhabitants reside in rural settings, mainly practicing subsistence farming and informal trade. The area is highly endemic for Plasmodium falciparum malaria with perennial transmission and two seasonal peaks coinciding with the short and long rains [6]. HIV sero-prevalence among antenatal clinic attendees was about 7% in 2007 [7].

The Tanzanian Expanded Programme of Immunization includes the following: Bacille Calmette-Guérin, live oral polio, diphtheria-whole cell pertussis-tetanus-hepatitis B and monovalent measles vaccines for children, as well as supplemental tetanus toxoid vaccine for women of child-bearing age. Tanzania had just started immunization against Haemophilus influenzae type b in March 2009 and hopes to introduce pneumococcal vaccine in 2010 [8]. Typhoid vaccine is not routinely administered in the county.

Fever Surveillance Procedures

Prior to the start and during the course of the study, emergency triage and hospital care guidelines were implemented in the ward [9]. On admission, children aged 2 months to 14 years were screened for eligibility during the study hours from 7am to 7pm, Monday-Sunday. Children with fever of 3 or more days prior to admission or fever of less than 3 days but with at least one severity criterion (respiratory distress, deep breathing, severe pallor with respiratory distress, prostration, capillary refill ≥3 seconds, temperature gradient, systolic blood pressure <70 mm Hg, coma, severe jaundice, history of 2 or more convulsions in last 24 hours, hypoglycemia, neck stiffness, bulging fontanel or desaturation) were recruited into the study. All clinical information was recorded in a standard case record form. Treatment was provided as per national guidelines. Outcome was recorded at discharge or death in a discharge form. Surveillance procedures were supervised by experienced study physicians (GM, IH, JD).

Point of Care and Laboratory Investigations

We collected 1 to 10 millilitres of blood (depending on body weight) from each eligible child. Immediate bedside testing included those for hemoglobin concentration (HemocueTM, Ängelholm, Sweden) and blood glucose level (Accu-checkTM, Roche Diagnostics GmbH, Germany). We performed two types of rapid diagnostic test (RDT) for P. falciparum malaria: HRP-2 based (ParachekTM, Orchid BioMedical, Mumbai, India or ParahitTM, Span Diagnostics, Surat, India) and LDH based (OptiMAL-IT, DiaMed AG, Switzerland). From each child, thin and thick blood films were prepared, Giemsa-stained and read by experienced laboratory technicians. At least 100 high power microscopic fields of the thin film were examined to exclude the diagnosis of malaria. Blood for culture was inoculated into a BacALERTTM Pediatric-fan bottle (bioMérieux, Marcy l’Etoile, France) and incubated in the BacT/ALERT 3D automated microbial detection system. Blood cultures were processed according to standard methods. Colonies with biochemical reactions on API20E, suggestive of Salmonella were confirmed serologically by slide and tube agglutination testing using specific O and H antisera (Becton Dickinson, NJ, USA). Sera were tested for the presence of HIV-1 and HIV-2 antibodies according to the National HIV rapid testing algorithm [10] using Capillus HIV-1, HIV-2 test (Trinity Biotech, Bray, Ireland) or SD Bioline (Standard Diagnostics, Kyonggi-do, Korea) followed by Determine HIV-1/2 test (Abbott Laboratories, IL, USA) if the first test was positive. Discordant results were resolved by a third antibody test, Unigold (Trinity Biotech, Bray, Ireland), which if positive rendered the sample as positive and if negative, the sample was considered as negative. Children aged less than 18 months with positive results were not tested by polymerase chain reaction for viral antigen and for that reason were excluded in the final analysis.

Data Management, Definitions and Analysis

Data were double-entered into custom-made data entry programs using MS-Access (Microsoft Corp, VA, USA). Data management programs included error, range and consistency check programs.

Fever was defined as history of a rise in body temperature as recalled by a care-giver or presence of axillary temperature ≥37.5°C on presentation. Bacteremia was defined as fever with isolation of pathogenic bacteria from blood culture, further classified as those caused by S. typhi (typhoid fever), NTS, and other (non-Salmonella) pathogenic bacteria. Malaria was defined as fever with a positive RDT or blood film. HIV infection was defined as a positive Capillus test or SD bioline, confirmed either by a positive Determine HIV-1/2 or a positive Unigold test. Low maternal education was considered as schooling to less than the National Curriculum standard 7. Diarrhea was defined as loose or watery stools ≥3 times per day. A seizure was regarded as abnormal movements with altered consciousness. Desaturation was defined as oxygen saturation less than 90% measured by pulse oximetry. Acute severe malnutrition was defined as the presence of bilateral pedal edema or severe wasting. We also assessed the mid-upper arm circumference (MUAC) of children between 12 to 59 months of age. Signs of shock were temperature gradient in the lower extremities and delayed capillary refill of ≥3 seconds or systolic blood pressure <70 mm Hg. Signs of dehydration were delayed skin pinch >2 seconds or sunken eyes. Prostration was defined as inability to sit unsupported (for children over 9 months of age) or to drink/breastfeed. Coma was defined as Blantyre coma score ≤2 for children less than 2 years of age or a Glasgow coma score ≤10 for older children. Hypoglycemia was defined as blood glucose level of <2.5 mmol/litre. Anemia was defined as hemoglobin of <8 g/dl and severe anemia <5 g/dl.

To assess potentially important distinguishing factors, we classified the cases into 5 non-mutually-exclusive groups: typhoid fever, invasive NTS infection, other pathogenic bacteremia, malaria, and those without bacteremia and malaria. Comparisons of categorical data were made using the Chi square or Fishers’ Exact test, as appropriate. Comparisons of continuous data were made using student’s t-test for data with equal variance or Welch’s t-test for those with unequal variance. All analyses were performed using StataTM v 10.0 (Stata Corp., TX, USA).

Literature Review

We conducted a literature review to compare our main findings with those from previous studies. Potential articles for inclusion were identified by direct searches of the MEDLINE database through PubMed. We included facility-based studies of children ≤16 years in sub-Saharan Africa that reported case fractions of S. typhi and NTS infection from sterile-site specimen (blood, CSF, lung or joint aspirate) bacterial cultures. The searches were restricted to publications from 1987 to date. For study sites with several publications generated from the same study population, only one citation was included unless the time period varied. We also conducted supplementary searches of the references in retrieved articles. Abstracts were reviewed and if relevant, the article was included.

Ethics

The fever surveillance was conducted following the principles governing biomedical research involving human subjects. Prior written informed consent was obtained from the parent or guardian of each eligible child. Pre-test counseling was provided
before HIV testing in accordance with local guidelines. The study was approved by the National Institute for Medical Research, Tanzania (NIMR/HQ/R.8a/Vol.IX/666) and the International Vaccine Institute - Institutional Review Boards, South Korea (IRB# 2007-017).

Results

From 1 March 2008 to 28 February 2009, 2319 children were admitted to Teule Hospital during study hours (Figure 1). After excluding 817 (35%) children who did not fulfill inclusion criteria or whose parents declined to participate in the study, 1,502 (65%) children were enrolled. Bacteria were isolated from the blood of 298 (20%) of these children, of which 142 (10%) were considered as likely contaminants. Each of the 156 children with pathogenic bacteremia had only a single organism isolated from their blood culture. Of the 156 (10%) bacteremic children, 14 (9%) had S. typhi, 45 (29%) had NTS and 97 (62%) had other pathogenic bacteria. Thus, invasive salmonellosis accounted for 59/156 (38%) bacteremic children.

We compared our findings with those from previous studies among children in sub-Saharan Africa (Table 1). Sixteen articles from 8 countries fulfilled our selection criteria [11–26]. In this series, the isolation rates of pathogenic bacteria ranged from 1 to 46%, the case fraction of S. typhi ranged from 0 to 42% and of NTS from 9 to 84%, depending on the study sampling frame. The number of NTS isolates for every one S. typhi ranged from 0.8 to 166.

Of the 1,502 children enrolled, 806 (54%) were between 2 months to 2 years, 520 (34%) were between 2 years to 5 years and 176 (12%) were >5 years of age (Table 2). We ranked the bacterial pathogenic isolates

![Figure 1. The study population.](doi:10.1371/journal.pone.0009244.g001)
| Country (urban or rural population) | Author, year (Reference number) | Study time frame | Age group | Sampling criteria | Number (specimen type) | Pathogenic bacteria (%) | S typhi (%) of pathogenic bacteria | Non-typhoidal salmonellae (%) of pathogenic bacteria | Number of NTS isolates for every 1 S typhi |
|-----------------------------------|-----------------------------------|------------------|-----------|------------------|------------------------|------------------------|--------------------------|--------------------------------------|----------------------------------|
| Tanzania (rural)                  | This paper                        | 2008–2009        | 2 months to 14 years | Admitted with ≥3 days fever or <3 days fever but with severity criteria | 1502 children (blood) | 156 (10) | 14 (9) | 45 (29) | 3 |
| 1. Gambia (rural)                 | Enwere et al. 2006 (11)           | 2003–2004        | 2–29 months | In and outpatients with signs of infection and a temperature of ≥38°C. Carried out as part of a pneumococcal vaccine trial | 7369 specimens (blood, CSF, lung aspirate) | 330 (4) | 0 (0) | 92 (28) | No S typhi |
| 2. Gambia (rural)                 | O’Dempsey et al. 1994 (12)        | 1989–1991        | <5 years | Admitted with pneumonia, meningitis or suspected sepsicaemia | 1162 children (blood) | 184 (16) | 11 (6) | 19 (10) | 2 |
| 3. Gambia (urban)                 | Mabey et al. 1987 (13)            | 1979–1984        | children  | Admitted (blood) | 247 | 45 (18) | 71 (29) | 2 |
| 4. Kenya (rural)                  | Williams et al. 2009 (14)         | 1998–2008        | <14 years | All admitted except those with elective procedures or accidents | 38441 children (blood) | 2157 (6) | 9 (0.4) | 211 (10) | 23 |
| 5. Kenya (rural)                  | Brent et al. 2006 (15)            | 2003             | <5 years | Randomly selected 10% of outpatients, excluding those admitted to hospital within the previous 10 days | 1093 children (blood) | 22 (2) | 0 (0) | 2 (9) | No S typhi |
| 6. Kenya (rural)                  | Berkley et al. 2005 (16)          | 1998–2002        | <13 years | All admitted except those with elective procedures or accidents | 19339 children (blood) | 228 (13) | 1 (0.4) | 166 (73) | 166 |
| 7. Kenya (rural)                  | Berkley et al. 1999 (17)          | 1993–1996        | children | Admitted with severe malaria | 783 children (blood) | 42 (5) | 0 (0) | 6 (14) | No S typhi |
| 8. Malawi (mixed)                 | Bronzan et al. 2007 (18)          | 1996–2005        | 6 months–15 years | Admitted with severe malaria | 1388 children (blood) | 64 (5) | 1 (2) | 37 (58) | 37 |
| 9. Malawi (mixed)                 | Walsh et al. 2000 (19)            | 1996–1997        | ≤15 years | Admitted with suspected bacteremia (febrile or very ill without an adequate explanation by physical examination or blood film) or who remained febrile after treatment for malaria | 2123 children (blood) | 365 (17) | 15 (4) | 140 (38) | 9 |
| 10. Mozambique (rural)            | Sigauque et al. 2009 (20)         | 2001–2006        | <15 years | All admitted <2 years of age. Those 2–14 years with temperature ≥39°C or with severity criteria | 19 896 children (blood) | 1550 (8) | 3 (0.2) | 401 (26) | 134 |
| 11. Nigeria (urban)               | Falade et al. 2009 (21)           | 2005–2006        | 2–59 months | Admitted with features of community-acquired pneumococcal disease | 330 children (blood) | 95 (29) | 0 (0) | 15 (16) | No S typhi |
according to frequency. Overall and among children less than 5 years of age, NTS was the principal organism. \textit{S. typhi} was the most common isolate among those over 5 to 14 years of age. Other commonly isolated bacterial pathogens included \textit{Escherichia coli} (27/156 or 17%) and \textit{Haemophilus influenzae} (20/156 or 13%). There were 8 (5%) \textit{Streptococcus pneumoniae} common isolate among those over 5 to 14 years of age. Other pathogenic bacteria (Figure). Considering those with a positive RDT or not, children with invasive NTS infection were more likely to also have malaria (33/45 or 73%) compared to those with typhoid fever (3/14 or 21%, p value 0.01). Severe palmar pallor, anemia and hypoglycemia were common among NTS infection, other pathogenic bacteremia, and malaria cases but those with invasive NTS had the lowest mean hemoglobin level (5.7 g/dl) and were most frequently hypoglycemic (6/45; 21%). Children with invasive NTS died more frequently (11/45; 24%; p value <0.01) compared to all other groups.

### Discussion

In our study population of children between 2 months to 14 years of age, \textit{Salmonella} ranked as the most common cause of bacteremia. Our over-all isolation rate of pathogenic bacteria was similar to that in previous studies that used similar sampling criteria [16,20]. But reviewing previous reports, we noted considerable variation in the case fraction of \textit{S. typhi} and NTS infections [11–26]. Other important lessons were gleaned from our review of the literature. First, we found that data was available only from a few sub-Saharan African countries with the majority of studies having been conducted in research centres in the Gambia, Kenya, Malawi, and Mozambique. Second, in all but one report [22], NTS outnumbered \textit{S. typhi} infections by several-fold. In many sites, pediatric typhoid fever was not detected at all. This is in marked contrast to findings from Asia where a high burden of disease is seen

| Country (urban or rural population) | Author, year (Reference number) | Study time frame | Age group | Sampling criteria | Number (specimen type) | Pathogenic \textit{S typhi} (%) | NTS isolates for every 1 \textit{S typhi} |
|-----------------------------------|----------------------------------|------------------|-----------|-------------------|------------------------|-----------------------------|----------------------------------|
| 12. Rwanda (mixed)                | Lepage et al. 1987 (22)          | 1984–1985        | <15-years | Outpatients with temp \(\geq 39^\circ C\) excluding those admitted to hospital within the preceding 3 months and those with measles up to 10 days after onset of rash | 140 children (blood) | 112 (1) | 47 (42) | 36 (32) | 0.8 |
| 13. Uganda (urban)               | Bachou et al. 2006 (23)          | 2003–2004        | <5-years  | Admitted with severe malnutrition | 445 children (blood) | 76 (17) | 5 (7) | 28 (37) | 6 |
| 14. Zaire (rural)                | Bahwere et al. 2001 (24)         | 1989–1990        | All children | On admission (whether febrile or not) | 779 children (blood) | 124 (16) | 2 (2) | 53 (43) | 27 |
| 15. Zaire (rural)                | Cheesbrough et al. 1997 (25)     | 1990–1992        | 1–16-years | In and outpatients, fitted into a preset clinical case definition of salmonella bacteraemia | 120 children (blood) | 55 (46) | 11 (20) | 35 (63) | 3 |
| 16. Zaire (rural)                | Green et al. 1993 (26)           | =5-years         |          | Admitted with clinically suspected salmonella infection (i.e. persistent fever, no response to anti-malarial treatment) | — (blood, CSF, joint aspirate) | 206 | 34 (17) | 172 (84) | 5 |

Table 1. Continued.

---

**Table 1.**

| Country (urban or rural population) | Author, year (Reference number) | Study time frame | Age group | Sampling criteria | Number (specimen type) | Pathogenic \textit{S typhi} (%) | Non-typhoidal salmonellae (%) | Number of NTS isolates for every 1 \textit{S typhi} |
|-----------------------------------|----------------------------------|------------------|-----------|-------------------|------------------------|-------------------------------|-----------------------------|----------------------------------|
| 12. Rwanda (mixed)                | Lepage et al. 1987 (22)          | 1984–1985        | <15-years | Outpatients with temp \(\geq 39^\circ C\) excluding those admitted to hospital within the preceding 3 months and those with measles up to 10 days after onset of rash | 140 children (blood) | 112 (1) | 47 (42) | 36 (32) | 0.8 |
| 13. Uganda (urban)               | Bachou et al. 2006 (23)          | 2003–2004        | <5-years  | Admitted with severe malnutrition | 445 children (blood) | 76 (17) | 5 (7) | 28 (37) | 6 |
| 14. Zaire (rural)                | Bahwere et al. 2001 (24)         | 1989–1990        | All children | On admission (whether febrile or not) | 779 children (blood) | 124 (16) | 2 (2) | 53 (43) | 27 |
| 15. Zaire (rural)                | Cheesbrough et al. 1997 (25)     | 1990–1992        | 1–16-years | In and outpatients, fitted into a preset clinical case definition of salmonella bacteraemia | 120 children (blood) | 55 (46) | 11 (20) | 35 (63) | 3 |
| 16. Zaire (rural)                | Green et al. 1993 (26)           | =5-years         |          | Admitted with clinically suspected salmonella infection (i.e. persistent fever, no response to anti-malarial treatment) | — (blood, CSF, joint aspirate) | 206 | 34 (17) | 172 (84) | 5 |
even in the youngest age groups [27,28]. Third, although NTS was isolated in all studies, there was a wide range in the case fractions by geographic location and time period. For example, in Kilifi, the case fraction of NTS decreased from 73% (1998 to 2002) to 10% (1998–2008) [14,16]. Interestingly, the burden of malaria in the area decreased substantially during this time period. It was estimated that hospital admissions for malaria decreased from 18-4 per 1000 children in 2003 to 3-4 in 2007 [29].

Malaria has long been suspected to increase the risk and contribute to the seasonality of invasive non-typhi salmonellosis [3]. The common occurrence of severe NTS septicemia during malaria outbreaks was first reported in British Guiana in the 1920’s [30]. Duggan and Beyer suggested an association between invasive salmonellosis and malaria in Nigerian children [31]. In 1987, Mabey et al found that young Gambian children with NTS septicaemia were more anemic and more likely to have evidence of recent malaria than were children of the same age with other forms of septicaemia [13]. In Malawi, studies have shown an association between NTS bacteraemia and severe anaemia [4,18,32]. In Kenya, Brent et al subsequently found that three-fourths of NTS patients with anaemia had evidence of either current or recent malaria [33]. In this study, NTS infections were significantly associated with prior antimalarial treatment and malarial complications (severe anaemia, jaundice and hypoglycaemia). And similar to a previous report [33], we found that a positive RDT with a negative blood smear for malaria was most common among the NTS group, suggesting a past rather than a current malaria infection. This supports the long-held hypothesis [13] that malaria is the preceding event which predisposes these children to invasive NTS infection. The mechanism underlying the association between malaria and NTS is incompletely understood. It is possible that the metabolic, haemodynamic or inflammatory processes that can occur during severe malaria also predispose to invasive bacterial disease [3]. Malnutrition was associated with NTS bacteremia among children in Kenya [33] but we could not confirm this in our study. We were also unable to explore other risk factors that have been associated with NTS infections in Africa such as contaminated food and water, animal contact, sickle cell disease, schistosomiasis, and recent antimicrobial use [3].

In this study, typhoid fever cases were more common in older children and presented with a longer duration of fever. In contrast, the non-typhoid bacteremia cases, as well as malaria, tended to occur in young children, particularly of poorly-educated women. It is likely that low educational attainment is a marker for low socioeconomic status. NTS infection was associated with a considerable increased risk for death.

Our study has several limitations. First, it is well known that blood cultures are insensitive for detecting bacteremia. Small blood volumes for culture especially from the younger and sicker patients, as well as the prior use of antibiotics, further decrease the sensitivity of blood cultures. Among our participants, 17% admitted to antibiotic use prior to admission. Thus, it is likely that low educational attainment is a marker for low socioeconomic status. NTS infection was associated with a considerable increased risk for death.

### Table 2. Bacterial species isolated from 156 children with bacteremia, ranked* according to frequency.

| Gram-positive | >2 y to 2 y (n = 806) Rank | >2 y to 5 y (n = 520) Rank | >5 y (n = 176) Rank | Total (%) (n = 1,502) Overall rank |
|---------------|--------------------------|--------------------------|-------------------|----------------------------------|
| -Streptococcus pneumoniae | 4 | 5 | 4 | 0 | 8 | 8 (5.1) | 8 |
| -beta haemolytic Streptococci, Group A & C | 5 | 4 | 2 | 9 | 2 | 6 | 9 (5.8) | 7 |
| -Staphylococcus aureus | 2 | 9 | 0 | 10 | 3 | 2 | 5 (3.2) | 10 |
| **Gram-negative** | | | | | | |
| -Salmonella typhi | 1 | 10 | 4 | 4 | 9 | 1 | 14 (9.0) | 4 |
| -Nontyphoidal salmonella species | 30 | 12 | 7 | 0 | 8 | 20 (12.8) | 3 |
| -Haemophilus influenzae (type B) | 17 | 3 | 7 | 7 | 0 | 8 | 27 (17.3) | 2 |
| -Escherichia coli | 21 | 2 | 5 | 2 | 1 | 7 | 6 (3.8) | 9 |
| -Acinetobacter species | 3 | 7 | 3 | 7 | 0 | 8 | 11 (7.1) | 5 |
| -Non-fermenters | 4 | 5 | 4 | 4 | 3 | 2 | 11 (7.1) | 5 |
| Other** | 3 | 7 | 5 | 2 | 3 | 2 | | |
| **Total pathogenic bacteria** | 90 | 42 | 24 | 156 (100) |
| **Contaminants*** | 94 | 38 | 10 | 142 |
| **Total** | 184 | 80 | 34 | 298 |

*Rank (by age group and overall) was the same for organisms with the same frequency.

**Species included: Candida (n = 1), Citrobacter braakii (n = 1), Haemophilus parainfluenzae (n = 2), Pantoea species (n = 1), Gram negative rods not identified (n = 6).

***Species included: Bacillus (n = 19), Diphtheroids (n = 6), Micrococcus (n = 6), alpha-hemolytic Streptococcus viridans (n = 3), coagulase negative Staphylococcus (n = 98), yeasts (n = 5), mixed bacterial species (n = 4), Gram positive rods not identified (n = 1).

doi:10.1371/journal.pone.0009244.t002
In summary, we found that in a malaria endemic region in Tanzania, invasive salmonellosis is an important cause of hospitalized febrile diseases among children. Invasive NTS disease is associated with a high risk for death. Certain suggestive clinical features (such as older age and long duration of fever for typhoid whereas concomitant malaria, anemia, jaundice and hypoglycemia for NTS infection) may be used to distinguish invasive salmonellosis from other severe febrile illness on presentation. We shall continue our fever surveillance in Teule hospital to follow trends in the occurrence and clinical picture of these infections in our community.

Table 3. Clinical features on admission and outcome of febrile cases, by non-mutually exclusive laboratory-confirmed groups.

|                      | All       | Group 1: Typhoid fever (n = 14) | Group 2: Invasive NTS infection (n = 45) | Group 3: Other pathogenic bacteraemia (n = 97) | Group 4: Malaria (n = 947) | Group 5: No bacteraemia and malaria (n = 474) | P value (1vs5) | P value (2vs5) | P value (3vs5) | P value (4vs5) |
|----------------------|-----------|---------------------------------|------------------------------------------|-----------------------------------------------|--------------------------|-----------------------------------------------|----------------|----------------|----------------|----------------|
| Mean age in years; n | 1,502     | 2.6                             | 7.5                                      | 2.0                                           | 2.3                      | 2.7                                           | 2.1            | 0.00           | 0.64           | 0.60           | 0.00           |
| N (%) male; n = 1,502| 813       | 54.1                            | 21.4                                     | 53.3                                          | 45.6                     | 501                                           | 52.9           | 574            | 517            | 578            | 0.01           |
| N (%) with low maternal education; n = 1,348 | 457       | 33.9                            | 30.0                                     | 43.6                                          | 41.4                     | 314                                           | 37.1           | 118            | 118            | 127            | 1.00           |
| Mean days of fever; | 1,499     | 5.0                             | 10.1                                     | 6.6                                           | 5.3                      | 4.4                                           | 5.7            | 0.02           | 0.45           | 0.47           | 0.00           |
| N (%) with cough; n | 1,502     | 876                             | 58.3                                     | 57.1                                          | 73.3                     | 66                                             | 68.0           | 490            | 517            | 333            | 0.37           |
| N (%) with diarrhea; n = 1,493 | 287       | 19.2                            | 6.2                                      | 42.9                                          | 10.2                     | 18                                             | 18.8           | 129            | 137            | 137            | 0.37           |
| N (%) with vomiting; n = 1,491 | 737       | 49.4                            | 10                                       | 71.4                                          | 24.3                     | 53                                             | 42.7           | 466            | 469            | 234            | 0.17           |
| N (%) with seizures; n = 1,495 | 220       | 14.7                            | 0                                        | 0                                             | 2.4                      | 15                                             | 15.5           | 180            | 191            | 72             | 0.61           |
| N (%) with coma; n = 1,476 | 94        | 6.4                             | 0                                        | 0                                             | 2.4                      | 12                                             | 12.4           | 77             | 83             | 10             | 1.00           |
| N (%) received antimarial; n = 1,485 | 921       | 62.0                            | 8                                        | 61.5                                          | 35                       | 77                                             | 66.7           | 585            | 622            | 287            | 1.00           |
| N (%) received antimicrobial; n = 1,485 | 247       | 16.7                            | 2                                        | 14.3                                          | 9                        | 20                                             | 24             | 104            | 112            | 121            | 0.53           |
| Mean axillary temp on admission; n = 1,483 | 38.1      | 38.5                            | 38.1                                     | 38.2                                          | 38.1                     | 38.0                                           | 0.07           | 0.68           | 0.15           | 0.10           | 0.00           |
| Mean heart rate on admission; n = 1,465 | 155.0     | 122.6                           | 163.0                                    | 153.6                                         | 156.6                    | 153.0                                          | 0.00           | 0.02           | 0.86           | 0.02           | 0.00           |
| N (%) with desaturation; n = 1,475 | 85        | 5.8                             | 1                                        | 7.7                                           | 3                       | 6.8                                            | 5.2            | 43             | 46             | 37             | 0.10           |
| N (%) with severe palmar pallor; n = 1,498 | 434       | 29.0                            | 0                                        | 0                                             | 28                       | 62                                             | 33             | 383            | 405            | 34             | 0.61           |
| Among children 12–59 months, n (%) with MUAC <12.5 cm; n = 859 | 47       | 3.5                             | 0                                        | 3                                             | 12.5                     | 2                                             | 4.2            | 16             | 28             | 13.3           | 1.00           |
| N (%) with sign of severe malnourishment; n = 1,499 | 47        | 3.1                             | 0                                        | 2                                             | 4.5                      | 5.2                                            | 14             | 1.5            | 27             | 5.7            | 1.00           |
| N (%) with sign of shock; n = 1,495 | 55        | 3.7                             | 0                                        | 0                                             | 2                        | 4.5                                            | 7             | 7.3            | 35             | 37             | 13             |
| N (%) with sign of dehydration; n = 1,459 | 60        | 4.1                             | 0                                        | 0                                             | 4                        | 9.1                                            | 5.4            | 18             | 19             | 36             | 0.61           |
| N (%) with jaundice; n = 1,475 | 18         | 1.2                             | 0                                        | 0                                             | 3                        | 6.8                                            | 1.1            | 14             | 1.5            | 3               | 1.00           |
| N (%) prostrated; n = 1,292 | 337       | 26.1                            | 2                                        | 20.0                                          | 7                        | 17.5                                           | 32             | 269            | 324            | 52             | 0.63           |
| N (%) with neck stiffness or bulging fontanelle; n = 1,491 | 24        | 1.6                             | 0                                        | 0                                             | 1                        | 2.2                                            | 5             | 5.2            | 6               | 6.0            | 13             |
| N (%) with impaired consciousness; n = 1,481 | 68        | 4.6                             | 1                                        | 7.1                                           | 3                        | 6.8                                            | 8             | 8.3            | 57             | 61             | 7               |
| Mean blood glucose in mmol/litre; n = 840 | 5.4       | 3.8                             | 4.5                                      | 4.8                                           | 5.3                      | 5.5                                            | 5.5            | 0.06           | 0.01           | 0.01           | 0.20           |
| N (%) with hypoglycemia; n = 840 | 63        | 7.5                             | 25.0                                     | 6                                             | 21.4                     | 7                                             | 12.3           | 48             | 7.8            | 8.4            | 0.18           |
| Mean hemoglobin in g/dl; n = 1,478 | 8.0       | 10.2                            | 5.7                                      | 7.9                                           | 7.1                      | 9.7                                            | 0.47           | 0.00           | 0.00           | 0.00           | 0.00           |
| N (%) with anemia; n = 1,478 | 674       | 45.6                            | 2                                        | 14.3                                          | 82                      | 45                                             | 46.9           | 550            | 593            | 95             | 0.75           |
| N (%) with severe anemia; n = 1,478 | 274       | 18.5                            | 0                                        | 0                                             | 40.0                     | 15                                             | 15.6           | 244            | 263            | 23             | 1.00           |
| Among children ≥18 months, n (%) HIV infected; n = 854 | 67        | 7.8                             | 1                                        | 8.3                                           | 2                        | 9.1                                            | 10             | 20.8           | 36             | 5.7            | 24             |
| Died; n = 1,502 | 92        | 61.1                            | 1                                        | 7.1                                           | 11                       | 24                                             | 11             | 113            | 54             | 5.7            | 27             |

*Comparisons of categorical data were made using the Chi square or Fishers' Exact test, as appropriate. Comparisons of continuous data were made using student's t-test for data with equal variance or Welch's t-test for those with unequal variance.

doi:10.1371/journal.pone.0009244.t003
Acknowledgments

The study is published with the permission of the Director General of the Tanzanian National Institute for Medical Research, Dar-es-Salaam. We are grateful to the patients and their parents who made this work possible. We thank all technical staff and research assistants who were involved in clinical and laboratory data collection: Aikande Shoo, Celina Antony Wychilfe, Revogit Tse, Weston Lemanya, Christina Kiemi, Emmanuel Sowai, Edward Milil, Wali Mbuya, Selemani Mtumgija, Marwa Msikwabe, Simphorosa Silaye, Stella Emmanuel, Rosalia Marwa, Regina Malugu and Michael Mgomea.

References

1. Graham SM (2002) Salmonellosis in children in developing and developed countries and populations. Curr Opin Infect Dis 15: 307–312.
2. Mwebi E, English M (2008) Typhoid fever in children in Africa. Trop Med Int Health 13: 352–354.
3. Morpeth S, Ramathatini HO, Crump JA (2009) Invasive Non-Typhoid Salmonella Disease in Africa. Clin Infect Dis 49: 606–611.
4. Graham S, Molyneux EM, Wallah A, Cherscheis J, Molyneux ME, et al. (2000) Nontyphoidal Salmonella infections of children in tropical Africa. Pediatr Infect Dis J 19: 1189–1196.
5. Statistics NBo (2002) United Republic of Tanzania: National Census.
6. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, et al. (2003) Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malar J 2: 24.
7. Edmonds SM, Meadway J (2009) Getting Pregnant Women onto HAART for data management: DRK. Made a substantial review of the analysis and interpretation of data: AM. Involved in laboratory analysis: JK. Did the analysis and interpretation of data: DRK. Made a substantial review of the manuscript: JLDR.

Author Contributions

1. Graham SM (2002) Salmonellosis in children in developing and developed countries and populations. Curr Opin Infect Dis 15: 307–312.
2. Mwebi E, English M (2008) Typhoid fever in children in Africa. Trop Med Int Health 13: 352–354.
3. Morpeth S, Ramathatini HO, Crump JA (2009) Invasive Non-Typhoid Salmonella Disease in Africa. Clin Infect Dis 49: 606–611.
4. Graham S, Molyneux EM, Wallah A, Cherscheis J, Molyneux ME, et al. (2000) Nontyphoidal Salmonella infections of children in tropical Africa. Pediatr Infect Dis J 19: 1189–1196.
5. Statistics NBo (2002) United Republic of Tanzania: National Census.
6. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, et al. (2003) Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. Malar J 2: 24.
7. Edmonds SM, Meadway J (2009) Getting Pregnant Women onto HAART for data management: DRK. Made a substantial review of the analysis and interpretation of data: AM. Involved in laboratory analysis: JK. Did the analysis and interpretation of data: DRK. Made a substantial review of the manuscript: JLDR.