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**Background.** Invasive nontyphoidal *Salmonella* (iNTS) has emerged as a cause of bacteremia in African children and HIV-infected adults, which is associated with high mortality. Epidemiological data and burden of iNTS infections in resource-constrained settings are needed to better define preventive and curative strategies.

**Methods.** Blood and, if appropriate, cerebrospinal fluid, were collected from children <15 years of age with fever or severe disease admitted to the Manhiça District Hospital and cultured for NTS; isolates were then characterized.

**Results.** From January 2001 to December 2014, 41,668 of the 51,878 admitted children had a blood culture performed. Invasive NTS was isolated from 670 (1.6%) specimens collected from 41,668 patients; 69 (10.3%) died. *Salmonella enterica* subspecies enterica serovar Typhi or *Salmonella enterica* subspecies enterica serovar Paratyphi A or C were only isolated in 14 (0.03%) patients. A total of 460 of 620 (74.2%) NTS isolates serotyped were *Salmonella enterica* subspecies enterica serovar Typhimurium (45% [116/258] of which were multilocus sequence type 313). The incidence of iNTS was 61.8 (95% confidence interval, 55.4–68.9) cases per 100,000 child-years, being highest among infants (217.7 cases/100,000 child-years). The incidence of iNTS declined significantly (*P* < .0001) over time, but the case fatality ratio remained constant at approximately 10%. Antimicrobial resistance of iNTS against most available antimicrobials has steadily increased, with a predominance of multidrug-resistant strains.

**Conclusions.** The decreasing but still high incidence of iNTS, its high associated case fatality ratio, and the common detection of multidrug-resistant strains call for a need to improve treatment and prevention strategies for iNTS.

**Keywords.** *Salmonella*; bacteremia; burden of disease; incidence; invasive nontyphoidal *Salmonella*.

Nontyphoidal *Salmonella* (NTS) is a prominent cause of bacteremia among young African children and human immunodeficiency virus (HIV)–infected adults, and invasive NTS (iNTS) has been associated with a case fatality ratio of 20%–25% [1]. HIV, malnutrition, and malaria seem to be major predisposing factors for this infection in sub-Saharan Africa [1, 2]. A novel genotype of *Salmonella enterica* subspecies enterica serovar Typhimurium called multilocus sequence type (ST) 313 is an important cause of iNTS in sub-Saharan Africa [3]. Whole-genome sequencing shows 2 lineages of *Salmonella* Typhimurium ST313, which emerged in association with the HIV pandemic, and perhaps also in relation to widespread chloramphenicol use [4]. NTS is a common cause of pediatric bacteremia [5, 6], and an important cause of pediatric meningitis in some sub-Saharan African countries [7]. In such settings, mortality rates >50% despite antibiotic therapy have been described [8]. A factor complicating treatment of invasive NTS is the high prevalence of multidrug-resistant (MDR) strains [9, 10].

Epidemiological data on iNTS are sparse in sub-Saharan Africa. In Mozambique, NTS was a predominant cause of pediatric bacteremia [11] and HIV-infected...
MATERIALS AND METHODS

Study Setting and Population
The study was conducted by the Centro de Investigação em Saúde da Manhiça (CISM) at the MDH, the main referral health facility for Manhiça District, a rural area located 80 km north of Maputo, southern Mozambique. Manhiça has a subtropical climate, with a warm and rainy season (November to April) and a cool and drier season during the rest of the year. The district has an estimated population of 160,000 inhabitants. In this area, CISM has been running a continuous demographic surveillance system (DSS) since 1996, covering approximately 95,000 inhabitants by 2013 [14]. A full description of the geographical and sociodemographic characteristics of the study community has been presented elsewhere [15]. Of importance, HIV seroprevalence in the area is among the highest in the world (40% of the general adult population) [16].

CISM is adjacent to MDH, and since 1997, the hospital and CISM have jointly operated a 24-hour surveillance of all pediatric (<15 years of age) visits to the outpatient department and admissions to the wards. During the study period (January 2001–December 2014), trained clinical officers or medical doctors routinely recorded clinical signs and symptoms observed upon admission using standardized forms. A thick blood smear for malaria parasites was obtained by finger prick from children with axillary temperature ≥37.5°C or reported history of fever within the preceding 24 hours. HIV testing among admitted children was not a routine procedure. However, from 2006 onward, HIV tests were conducted in the context of specific studies, or to children with a clinical suspicion of immunosuppression according to the national guidelines with 2 rapid antibody tests (Determine, Alere, Chiba, Japan; and Unigold, Trinity Biotech, Co-Wicklow, Ireland, only when Determine was positive) or polymerase chain reaction (PCR) for children <9 months of age.

Definitions
Bacteremia was defined as the isolation of 1 or more noncontaminant bacteria from the blood culture on admission. Coagulase-negative Staphylococci, Bacillus species, or micrococci were classified as contaminants. NTS included Salmonella enterica subspecies enterica serovars other than Typhi and Paratyphi A, B, or C recovered from blood or cerebrospinal fluid (CSF).

An acute clinical malaria case was defined as a child admitted with a clinical diagnosis of malaria with confirmed *P. falciparum* asexual parasitemia >0 parasites/µL [17].

Mild anemia was defined as a packed cell volume (PCV) 25% to <33%, moderate anemia as a PCV 15% to <25%, and severe anemia as a PCV <15% on admission. Case fatality ratios (CFRs) were calculated considering children with known outcome at discharge, excluding patients who left the admission wards without medical permission or transferred to Maputo Central Hospital.

Nutritional status was assessed using weight-for-age z score, calculated using the LMS (Lambda for the skew, M for the median, and Sigma for the generalized coefficient of variation) method and the 2000 Centers for Disease Control and Prevention growth reference charts [18]. Acute bacterial meningitis was confirmed when the culture of CSF yielded a pathogen excluding contaminants or the CSF was purulent and either the results of blood culture were positive or results of the CSF Gram stain were positive [19].

Sample Collection and Laboratory Procedures
As part of routine clinical practice at MDH, a single venous blood specimen for bacterial culture was collected on hospital admission for all children <2 years of age, and for children 2 to <15 years with axillary temperature ≥39.0°C or with signs of severe illness as judged by the admitting clinician [11]. Between 1 mL and 3 mL of whole blood was collected from children meeting inclusion criteria and immediately inoculated into pediatric bottles (Pedibact; Becton-Dickinson, Franklin Lakes, New Jersey) and incubated in a continuously monitored blood culture system (Bactec 9050 or Bactec FX200; Becton-Dickinson, Sparks, Maryland) for 5 days. This procedure has been in place since May 2001; prior to May 2001, blood culture bottles were placed in incubators and checked for positivity at day 2 by Gram stain. Positive samples were plated in solid media, whereas negative samples were reincubated and reexamined by Gram stain at day 7. Positive blood cultures were examined by Gram stain and subcultured on solid media according to Gram stain findings.

Bacterial isolates were identified following standard microbiological procedures [20]. Serotyping to identify *Salmonella Typhimurium* and *Salmonella Enteritidis* were conducted at the CISM laboratory using commercially available slide agglutination antisera (Bio-Rad, Marnes-la-Coquette, France) [21]. Other *Salmonella* serovars were determined at the National Reference Laboratory of the Instituto de Salud Carlos III (ISCIII), Madrid, Spain, which also performed quality control checks of approximately 20% of isolates serotyped at CISM’s laboratory. ISCIII also phage-typed the isolates belonging to *Salmonella enterica* subspecies enterica serovars Typhimurium, Enteritidis, Hadar, and Virchow following World Health Organization recommendations. Sequence types of *Salmonella Typhimurium*
were determined by PCR [3] and confirmed by performing multilocus sequence typing [22] at the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore.

Isolates were tested for their susceptibility by standard disk diffusion method for ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, gentamicin, tetracycline, nalidixic acid, ciprofloxacin, ceftriaxone, and amoxicillin-clavulanic acid (Mast Group Ltd, Merseyside, United Kingdom). Interpretative categories of resistance were performed based on the Clinical and Laboratory Standards Institute guidelines [23] at CISM’s laboratory.

### Statistical Analysis

Admission standardized questionnaires were double-entered in FoxPro (version 2.6, Microsoft Corporation, Redmond, Washington) at CISM, and discrepancies in data entry were resolved by referring to the original forms. Statistical analyses were performed using Stata software (version 13.0, Stata Corporation, College Station, Texas). Proportions were compared using χ² tests, and odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression. Wilcoxon rank-sum tests were used for nonparametric comparisons. Minimum community-based incidence rates of *Salmonella* bacteremia and 95% CIs were calculated considering individual time at risk for children residing in the CISM study area excluding periods of migration. In calculating person-time, individuals were excluded during a lag period of 15 days after each episode of community-acquired bacteremia. Negative binomial regression models were estimated to compare incidence rates. Score test for trend of rates with calendar year was assessed by Mantel–Haenszel method to analyze the incidence over time of *Salmonella* and clinical malaria.

### RESULTS

#### Description of Study Population

During the study period (January 2001–December 2014), 51,878 children aged <15 years were admitted to the MDH; blood cultures were collected in 41,668 (80.3%), and 670 (1.6%) were NTS. *Salmonella Typhimurium* and *Salmonella Enteritidis* accounted for 95.6% of all serotyped strains. There were only 14 typhoidal *Salmonella* isolates (7 *Salmonella Typhi*, 6 *Salmonella Paratyphi C*, and 1 *Salmonella Paratyphi A*). In the same period, 3893 patients had a lumbar puncture performed to rule out meningitis, but NTS could only be confirmed in 1 (<0.1%) case.

Of iNTS isolates, 365 (54.6%) were recovered from males, and the mean age of children with NTS bacteremia was 21.5 months (SD, 22.7 months). Nutritional status was recorded for 589 NTS patients, of whom 183 (31%) were severely malnourished.

Children with iNTS were significantly more likely to have severe malnutrition (adjusted OR, 3.9 [95% CI, 3.1–5.0]), splenomegaly (adjusted OR, 3.9 [95% CI, 3.1–5.0]), and moderate or severe anemia (adjusted OR, 5.0 [95% CI, 3.5–7.2]) compared with children <15 years of age hospitalized during the study period without NTS bacteremia, and were less likely to have acute malaria (OR, 0.6 [95% CI, 0.5–0.8]). Table 1 summarizes the clinical features of children with NTS bacteremia vs children without NTS bacteremia.

#### Serotype, Phage Type, and Sequence Type Distribution

Fifty of the 670 (7.5%) NTS isolates were not serotyped. Of 620 *Salmonella enterica* isolates serotyped, 460 (74.2%) were *Salmonella Typhimurium*, 133 (21.5%) were *Salmonella Enteritidis*, while other serovars accounted for the remaining 4.4%
Table 2. Minimum Community Incidence Rates of Invasive Nontyphoidal Salmonella Serovars Among Children Living in the Manhiça Demographic Surveillance System Study Area, Mozambique, 2001–2014

<table>
<thead>
<tr>
<th>Serotypes and Age Group</th>
<th>Person-years</th>
<th>Episodes, No.</th>
<th>Incidence Rate/100 000 Children-years (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Salmonella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–11 mo</td>
<td>41,340.5</td>
<td>90</td>
<td>217.7 (177.1–267.7)</td>
</tr>
<tr>
<td>12–59 mo</td>
<td>11,384.0</td>
<td>201</td>
<td>172.7 (150.4–198.3)</td>
</tr>
<tr>
<td>≥60 mo</td>
<td>358,352.6</td>
<td>28</td>
<td>7.8 (5.4–11.3)</td>
</tr>
<tr>
<td>All ages</td>
<td>516,077.1</td>
<td>319</td>
<td>61.8 (55.4–68.9)</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–11 mo</td>
<td>41,341.6</td>
<td>60</td>
<td>145.1 (112.7–186.9)</td>
</tr>
<tr>
<td>12–59 mo</td>
<td>116,386.3</td>
<td>140</td>
<td>120.3 (101.9–142.0)</td>
</tr>
<tr>
<td>≥60 mo</td>
<td>358,352.9</td>
<td>22</td>
<td>6.14 (4.0–9.3)</td>
</tr>
<tr>
<td>All ages</td>
<td>516,080.8</td>
<td>222</td>
<td>43.0 (37.7–49.1)</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–11 mo</td>
<td>41,343.1</td>
<td>18</td>
<td>43.5 (27.4–69.1)</td>
</tr>
<tr>
<td>12–59 mo</td>
<td>116,390.2</td>
<td>38</td>
<td>32.7 (23.8–44.8)</td>
</tr>
<tr>
<td>≥60 mo</td>
<td>358,353.6</td>
<td>4</td>
<td>1.1 (0.4–3.0)</td>
</tr>
<tr>
<td>All ages</td>
<td>516,086.9</td>
<td>60</td>
<td>11.6 (9.0–15.0)</td>
</tr>
<tr>
<td>Other serovars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–11 mo</td>
<td>41,343.3</td>
<td>15</td>
<td>36.3 (21.9–60.2)</td>
</tr>
<tr>
<td>12–59 mo</td>
<td>116,390.9</td>
<td>23</td>
<td>19.8 (13.1–29.7)</td>
</tr>
<tr>
<td>≥60 mo</td>
<td>358,353.7</td>
<td>2</td>
<td>0.6 (1.2–2.2)</td>
</tr>
<tr>
<td>All ages</td>
<td>516,087.9</td>
<td>40</td>
<td>7.8 (5.7–10.6)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

Table 3. Trends of Case Fatality Ratio Among Children With Invasive Nontyphoidal Salmonella With Known Outcome

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of iNTS Cases</th>
<th>CFR, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>119</td>
<td>10 (8.4)</td>
</tr>
<tr>
<td>2002</td>
<td>110</td>
<td>9 (8.1)</td>
</tr>
<tr>
<td>2003</td>
<td>110</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>2004</td>
<td>66</td>
<td>16 (24.2)</td>
</tr>
<tr>
<td>2005</td>
<td>37</td>
<td>4 (10.8)</td>
</tr>
<tr>
<td>2006</td>
<td>61</td>
<td>8 (13.1)</td>
</tr>
<tr>
<td>2007</td>
<td>28</td>
<td>4 (13.1)</td>
</tr>
<tr>
<td>2008</td>
<td>36</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>2009</td>
<td>16</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>2010</td>
<td>12</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>2011</td>
<td>17</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>2012</td>
<td>19</td>
<td>3 (15.7)</td>
</tr>
<tr>
<td>2013</td>
<td>19</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>2014</td>
<td>13</td>
<td>1 (7.9)</td>
</tr>
<tr>
<td>Total</td>
<td>664</td>
<td>69 (10.3)</td>
</tr>
</tbody>
</table>

Abbreviations: CFR, case fatality ratio; iNTS, invasive nontyphoidal Salmonella.
performed, children infected with other serovars or serogroups than *Salmonella* Typhimurium or *Salmonella* Enteritidis were more likely to die (OR, 2.3 [95% CI, 1.19–4.57]; *P* = .014). Table 3 summarizes trends in CFR among children infected with NTS throughout the study period. 

In 2001 and 2002, *Salmonella* Typhimurium strains were mostly ST19 (93.8% and 75%, respectively). From 2003, ST313 emerged as an important strain, accounting for 28 of 45 (62.3%) of the strains serotyped, and peaking in 2007, with 14 of 16 (87.5%), whereas ST19 was found in only 2 (12.5%). Antimicrobial resistance data showed a high proportion of NTS strains resistant to chloramphenicol (299 [47.2%]) or ampicillin (428 [67.4%]). Table 4 shows antibiotic susceptibility of NTS isolated between 2001 and 2013 for the antimicrobials commonly used in the study area, Mozambique.
We were unable to demonstrate a major role for iNTS as a cause of acute bacterial meningitis in our setting. Indeed, throughout the study period, only 1 NTS was isolated from >3000 CSF cultures. We are unable to provide a clear explanation of the low incidence of iNTS meningitis in our setting, a surprising finding in relation to the high incidence of iNTS meningitis also documented in neighboring Malawi [7, 35], and this warrants more specific research.

Description of the specific circulating serovars is helpful to better define appropriate management and preventive strategies for iNTS, including the first-line antibiotic therapy recommendations or the development of vaccines. Subtyping is critical to understand strain diversity and differences in circulating strains’ virulence, something that may help understand the unchanged associated CFR throughout the study period despite the clear decline of incidence. The recent suggestion that the ST313 genotype may be more virulent than ST19, in relation to its incapacity of being adequately killed by macrophages [36], may support this hypothesis, particularly in the light of an increasing trend of this particular genotype in Manhiça. Not less important, the issue of antimicrobial resistance, increasing throughout the study period for iNTS isolates, is pressing in most developing countries such as Mozambique, due to the lack of second-line recommended antibiotics [37], which tend to be more costly.

Our data collectively reinforce the need for further investigations of iNTS across Africa to accurately measure the burden of iNTS disease and to improve treatment and prevention strategies (eg, vaccine development). The description of the most frequent iNTS serovars and associated incidence could help to estimate the proportion of infections that could be prevented or reduced with specific interventions against particular serovars if effective vaccines were available. These data have important implications for defining public health policy in Mozambique where the microbiological capacities are lacking and HIV, malaria, and malnutrition are prevalent and may fuel the spread of iNTS.

Notes

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