Invasive *Salmonella* Infections at Multiple Surveillance Sites in the Democratic Republic of the Congo, 2011–2014

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**Background.** This study reports the microbiological landscape of *Salmonella* Typhi and invasive nontyphoidal *Salmonella* (iNTS) in the Democratic Republic of the Congo (DRC).

**Methods.** Blood cultures obtained from hospital-admitted patients suspected of bloodstream infection (BSI) in 4 of 11 provinces in DRC (Kinshasa, Bas-Congo, Equateur, and Orientale) were processed. Sampling had started in 2007; the results for the period 2011–2014 are reported.

**Results.** *Salmonella* Typhi and iNTS were cultured from 194 (1.4%) and 840 (5.9%), respectively, of 14 110 BSI episodes and ranked first among BSI pathogens in adults (65/300 [21.7%]) and children (783/1901 [41.2%]), respectively. A total of 948 of 1034 (91.7%) isolates were available for analysis (164 *Salmonella* Typhi and 784 iNTS). *Salmonella* Typhimurium and *Salmonella* Enteritidis represented 386 (49.2%) and 391 (49.9%), respectively, of iNTS isolates, fluctuating over time and geography and increasing during the rainy season. Adults accounted for <5% of iNTS BSI episodes. Children <5 years accounted for 20.3% of *Salmonella* Typhi BSI episodes. Among *Salmonella* Typhi, rates of multidrug resistance and decreased ciprofloxacin susceptibility (DCS) were 37.8% and 37.2%, respectively, and 18.3% displayed combined multidrug resistance and DCS; rates of azithromycin and ceftriaxone resistance were 0.6% and absent, respectively. Among NTS isolates, ≥80% (79.7% of *Salmonella* Enteritidis and 90.2% of *Salmonella* Typhimurium isolates) showed multidrug resistance, and <2.5% showed DCS. Combined extended-spectrum β-lactamase production (*blaTEM-1* gene) and azithromycin resistance was noted in 12.7% of *Salmonella* Typhimurium isolates, appearing in Bas-Congo from 2013 onward.

**Conclusions.** *Salmonella* Typhi and NTS are major causes of BSI in DRC; their antimicrobial resistance is increasing.

**Keywords.** *Salmonella* Typhi; nontyphoidal *Salmonella*; bloodstream infection; antibiotic resistance.

The Democratic Republic of the Congo (DRC) is the third-largest country in Africa, encompassing 2 345 409 km² and extending from both sides of the equator (Figure 1). The climate is hot and humid, with a dry season during the periods December to February (north of the equator) and June to September (south of the equator) [1]. In 2012, its population was estimated at 77 800 000 inhabitants with an annual 3.4% increase; 52.0% of the population is aged <15 years and life expectancy is 56.5 years [2]. The majority (88%) of the population is living below the poverty line of US$1.25 per day [1]. In the 2014 ranking of the Human Development Index, DRC was listed second from last [3].

The prevalence of human immunodeficiency virus (HIV) infection in adults aged 15–49 years was 1.1% in 2013 [4]. More than 97% of the population is living...
in areas with high and stable malaria transmission, with >95% of Plasmodium infections caused by Plasmodium falciparum. With 14 000 000 malaria–confirmed infections in 2013, DRC (together with Nigeria) is the hardest-hit sub-Saharan country and cases have not been declining over recent years [5]. In a nationwide survey conducted in 2013, the prevalence of severe chronic and acute malnutrition among children <5 years of age was 43% and 8%, respectively [2].

Diagnostic microbiology facilities are nonexistent in DRC; only 8 of 536 (1.5%) health facilities surveyed in the capital, Kinshasa, reported performing blood cultures [6]. In partnership with the Institute of Tropical Medicine (ITM, Antwerp, Belgium), the National Institute of Biomedical Research (INRB) in Kinshasa started a network of microbiological surveillance of pathogens involved in bloodstream infections (BSIs) in 2007. A free-of-charge blood culture system was set up in the capital Kinshasa and later extended to sentinel hospitals in interior DRC; in addition, blood cultures were added to the tools used for microbiological assessment of suspected epidemics conducted by INRB. This article reports the results of blood cultures collected at selected healthcare facilities in DRC from 2011 to 2014, and describes the characteristics of the Salmonella enterica Typhi and of Salmonella enterica serotypes other than Typhi (nontyphoidal Salmonella [NTS] isolates), comprising 2 outbreaks of NTS, which have been described in detail elsewhere [7, 8]. Comparisons are made with the first surveillance period of the network (2007–2010) [9, 10].

**MATERIALS AND METHODS**

**Study Sites and Study Period**

The microbiological surveillance network of INRB comprised sentinel health facilities in 4 of 11 provinces of DRC that submitted blood cultures on a regular basis during the period January 2011–December 2014—namely, Kinshasa, Equateur, Orientale, and Bas-Congo (Figure 1). In addition, samples were obtained during outbreak investigations in the provinces Kasaï Occidental, Bandundu, and Maniema. In Kinshasa, main study sites comprised the university hospital of Kinshasa, 2 hospitals, and 6 healthcare centers with admission facilities belonging to both the public and the religion based healthcare sectors. In Equateur and Bas-Congo, samples were mainly obtained through the referral hospitals of Bwamanda and Kisantu, respectively. In province Orientale, sampling was coordinated by the University Hospital of Kisangani, which obtained samples from referral hospitals and private healthcare centers.

**Blood Cultures: Indications, Sampling, and Workup**

Blood specimens were routinely collected from patients >28 days of age admitted to the participating healthcare facilities with (1) a body temperature of ≥38°C or ≤35.5°C; (2) suspicion of severe localized infections (pneumonia, meningitis, complicated urinary tract infection, osteomyelitis and arthritis, severe skin and soft tissue infections, gynecological infections, and peritonitis), or (3) suspicion of sepsis, typhoid fever, and severe malaria. Additional criteria for collection of blood specimens from women in the neonatal period were premature rupture of membranes, intrapartum fever, low Apgar score, or specific symptoms such as tachypnea, cyanosis, and lethargy.

For children (≤14 years old), 1–4 mL of blood was sampled into a pediatric blood culture vial (BacT/ALERT PF, bioMérieux, Marcy L’Étoile, France); for adults, 2 × 10 mL of blood was inoculated into aerobic blood culture vials (BacT/ALERT FA, bioMérieux). Inoculated vials were processed on site (Hospital of Kisantu, University Hospitals of Kinshasa and Kisangani) or shipped to INRB. Bottles were incubated at 35°C and checked daily for growth by visual inspection of the chromogenic growth indicator; as a control, 5% of the blood cultures that did not grow were randomly selected and blinded subcultured. Grown bottles were Gram stained, subcultured to MacConkey and 5% sheep blood agar (Difo, Franklin Lakes, New Jersey), and incubated at 35°C for 24 hours. Isolates were identified to the species level by standard biochemical methods. Skin or environmental bacteria (coagulase-negative staphylococci, Corynebacterium species, Propionibacterium species, and Bacillus species) were categorized as contaminants; the other bacteria were considered as clinically significant organisms (CSOs) [11].

Colonies suspected of Salmonella were identified as Salmonella Typhi or NTS by their biochemical characteristics as
previously described [9, 10]. Isolates were stored in tubes of Tryptone Soya Agar (Oxoid, Basingstoke, United Kingdom) and shipped to ITM for confirmation and further workup.

**Successive Workup of Salmonella Isolates: Serotyping and Antibiotic Susceptibility Testing**

At ITM, isolates biochemically confirmed as *Salmonella* species were serotyped using commercial antisera (Sifin, Berlin, Germany). A random selection of 10% of isolates was sent to the National Reference Centre in Belgium for confirmation or extension of serotyping following the Kauffmann–White scheme [12]. Antibiotic susceptibility tests were performed in batch testing by disk diffusion (Neo-Sensitabs, Rosco, Taastrup, Denmark). The panel was selected according to the Clinical and Laboratory Standards Institute (CLSI) and supplemented with recommendations of the European Centre for Disease Prevention and Control [13, 14] (Supplementary Table 1). For ciprofloxacin and azithromycin, minimal inhibitory concentration (MIC) values were determined using the Etest macromethod (bioMérieux) or, in view of production and delivery problems at bioMérieux, M.I. C. Evaluator Strips (Oxoid). Extended-spectrum 

**Molecular Mechanisms of Antibiotic Resistance**

Molecular mechanisms of ciprofloxacin resistance were assessed by amplification and sequencing of the quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*, and *parC* genes as previously described [17, 18]. The presence of the plasmid-mediated quinolone resistance (PMQR) and β-lactamase genes was determined using polymerase chain reaction with newly designed primer pairs to cover a maximal number of currently described resistance genes (Supplementary Table 2).

**Data Registration and Statistical Analysis**

Data were encoded into an Excel database (Microsoft, Redmond, Washington). Isolates recovered from successive blood cultures drawn within 2 weeks after the initial one were considered as duplicates; those from repeat blood cultures drawn >2 weeks after the initial culture were considered as a separate (new) BSI episode. For analysis of antibiotic resistance data, only the isolate from the first BSI was considered. Statistical analysis was done with Stata software, version 12 (Stata Corp, College Station, Texas). Differences between proportions were tested for significance using the χ² test, and differences for median values by the Wilcoxon Mann–Whitney nonparametric test. A P value of <.05 was considered significant.

**Ethical Issues**

Ethical approval for the Microbiological Surveillance Study was granted by the Institutional Review Board of ITM, the Ethics Committee of Antwerp University, and the Ministry of Health of the DRC.

**RESULTS**

**Patient Characteristics**

From 2011 to 2014, blood specimens were collected from 14 110 patients suspected of BSI; 13 404 were unique patients. In total, 5591 (39.6%) BSI episodes occurred in children <2 years old.
and 759 (5.4%) were neonates. Adults accounted for 2490 (17.6%) of BSI episodes, of whom 192 (1.4%) were >65 years old.

**Clinically Significant Organisms and Proportions of *Salmonella***

Table 1 lists the number of suspected BSI episodes sampled and those grown with *Salmonella* for the different provinces during the study period. The total numbers of blood cultures sampled as well as the numbers of iNTS recovered showed an increase in 2014, associated with increased sampling in the provinces of Bas-Congo and Orientale. In Bas-Congo, an increase in iNTS cases was observed in 2014, whereas in province Orientale, the sampling sites were extended to the hospital of Buta, 350
km from the University Hospital of Kisangani. For the Kinshasa province, the numbers for the years 2012–2014 were lower than those from 2011 because health facilities with irregular submission or high contamination rates were excluded from 2012 onward.

Figure 2 displays the breakdown of blood cultures: overall, there was an 11.3% (1713/15 157) contamination rate, while in 16.7% (2353/14 110) suspected BSI episodes, blood cultures grew with CSOs. Proportions of CSOs were significantly higher in children compared with adults (16.4% [1901/11 619]) vs 12.0% [300/2490], respectively; \( P < .001 \% \). In adults, Salmonella Typhi ranked first among CSOs, immediately followed by E. coli; in children, iNTS were by far the most frequent CSOs. Of note, pneumococci accounted for only 0.2% (5/2353) of CSOs. In 3 patients (all <1 year of age), Salmonella Enteritidis was isolated from a second BSI episode on 22, 37, and 50 days after the first BSI.

Serotype Distribution According to Geography and Study Period
A total of 948 of 1034 (91.7%) Salmonella isolates were available for analysis. Salmonella Typhimurium and Salmonella Enteritidis each represented nearly half of iNTS isolates (386 [49.2%] and 391 [49.9%] of 784, respectively). The remaining isolates (n = 7) included the serotypes Aberdeen, Augustenborg, Colindale, Stanleyville, Salmonella group B, and Paratyphi C.

Figure 3 displays the distribution of the main serotypes over time and provinces. In 2014, the numbers of blood cultures sampled (Table 1) as well as iNTS recovered, increased markedly in the referral hospital of Kisantu, Bas-Congo. There was a seasonal tendency with higher numbers of iNTS isolates during the rainy season, particularly south of the equator (Figure 3). For Salmonella Typhi, this trend was less apparent.

Age and Sex Distribution
Age distribution of patients is shown in Table 2. The median age of patients infected with Salmonella Typhi was higher (\( P = .009 \)) vs those with an iNTS BSI, but 20.3% of patients with Salmonella Typhi BSIs were <5 years old. Age distributions for Salmonella Typhimurium and Salmonella Enteritidis infections were similar, with nearly half of affected patients being <2 years of age. Adult patients accounted only for a small proportion (<5%) of iNTS, with equal distribution over the age decades. The male-to-female ratio was approximately 1.2 for all serotypes, in line with that for the total number of suspected BSI episodes (7293/5458 = 1.3).

Antibiotic Resistance Profile
Table 3 lists the antibiotic resistance profiles for 940 isolates. Among Salmonella Typhi isolates, multidrug resistance and DCS rates were 37.8% and 37.2%, respectively; 30 of 61

![Figure 3. Over-time occurrence of main Salmonella serotypes in the Democratic Republic of the Congo (DRC) according to rainfall, north of the Equator (Bwamanda and Kisangani) and south of the Equator (Bas-Congo).](https://academic.oup.com/cid/article-abstract/61/suppl_4/S346/459038/435)

**Table 2. Age and Sex Distribution for the Main Serotypes of Salmonella enterica Isolates Recovered From Blood Cultures and Available for Further Workup**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Salmonella Typhi (n = 158a)</th>
<th>Salmonella Typhimurium (n = 377b)</th>
<th>Salmonella Enteritidis (n = 385b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>16.1 (10–23)</td>
<td>3.1 (0–1)</td>
<td>3.8 (1–3)</td>
</tr>
<tr>
<td>Age range</td>
<td>11 d–75 y</td>
<td>20 d–64 y</td>
<td>9 d–76 y</td>
</tr>
<tr>
<td>Aged &lt;15 y</td>
<td>63.3%</td>
<td>97.1%</td>
<td>94.8%</td>
</tr>
<tr>
<td>Aged &lt;5 y</td>
<td>20.3%</td>
<td>88.6%</td>
<td>82.9%</td>
</tr>
<tr>
<td>Aged &lt;2 y</td>
<td>5.1%</td>
<td>54.1%</td>
<td>52.2%</td>
</tr>
<tr>
<td>Aged &lt;28 d</td>
<td>0.01%</td>
<td>0.01%</td>
<td>0.01%</td>
</tr>
<tr>
<td>Male-to-female ratio</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Incomplete data (age and/or sex) were noted for 16, 11, and 20 isolates among Salmonella Typhi, Salmonella Typhimurium, and Salmonella Enteritidis, respectively. Numbers of Salmonella isolates for which complete data for age and sex were available.
(49.2%) of the DCS isolates were also multidrug resistant, and none of the isolates were fully resistant to ciprofloxacin (Table 3 and Supplementary Table 3). For Salmonella Typhi, DCS was associated with a single gyrA mutation in 55 of 61 (90.2%) of isolates, predominantly in residue Ser83 (n = 43) or Asp87 (n = 11); no mutations were found in the QRDR of gyrB and parC. All isolates were susceptible to ceftriaxone, and azithromycin resistance was <1%. The majority of both Salmonella Enteritidis and Salmonella Typhimurium isolates were multidrug resistant; multidrug resistance rates were higher among Salmonella Typhimurium vs Salmonella Enteritidis (90.2% and 79.7%, respectively; P < .001). Among both serotypes, DCS was <2.5%, with 14 of 16 isolates showing a single gyrA mutation in either residue Ser83 (n = 8) or Asp87 (n = 6). No mutations were found in the gyrB and parC genes. PMQR genes were identified in only 2 (2.4%) isolates (both serotypes). Of the 12 PMQR genes assessed, only qnrB1 (n = 1) and aac(6’)-Ib-cr (n = 1) were found. ESBL production was observed in 49 (12.7%) of Salmonella Typhimurium isolates. All ESBL-producing isolates were also azithromycin resistant, with MIC values extending to ≥256 mg/L.

**DISCUSSION**

The present study provides an overview of the patient profiles, serotype distribution, and antibiotic resistance profiles of Salmonella Typhi and iNTS at surveillance sites in DRC.

The present study had its limitations. Most important, the surveillance network aimed to generate microbiological information in preparation of implementing antibiotic stewardship programs in the different hospital settings. It was not designed to generate population incidence rates, and patient outcomes were not registered. Next, surveillance intensity differed between the sites, and was less regular for the Equateur province. Further, sampling and laboratory workup on site were done by local clinical and laboratory staff in addition to their routine daily tasks, which meant that missing data were not infrequent. Quality indicators were not optimal: The proportion
of contaminants (11.3%) exceeded both the target of 3% and the reported rates from resource-rich settings (>6%) [19]. In addition, the rate of CSOs (16.7%) was above the optimal range (6%–12%) [11], particularly when taking into account the malaria endemicity; this observation points to the possibility of too-restrictive sampling, that is, restricted to the patients in the worst clinical condition. The apparent absence of pneumococci can be at least partly explained by the high proportion of patients using antibiotics prior to sampling and the fact that transport to the laboratory in some cases took considerable time as a result of poor road infrastructure [1].

However, the surveillance system was embedded in the routine clinical setting for a longer time period (currently 6 years). Blood cultures were not charged to the patient, avoiding bias against difficult-to-treat patients. The healthcare facility–based design allowed the recovery of more isolates for analysis compared with outpatient-based studies [20].

During the first years of the surveillance network (period 2007–2010), Salmonella Typhi and iNTS grew from 1.9% and 3.2% of all suspected BSI episodes, vs 1.4% and 5.9% in the present series [6]. This difference may be partly explained by the fact that the surveillance network originally addressed adult patients suspected of typhoid fever and that reference hospitals with a large pediatric population were included only at a later stage. The geographic and over-time distribution reflected several local outbreaks, such as the increase of Salmonella Typhi in Kinshasa in 2014 as well as epidemic increases of iNTS in 2011 in the Bas-Congo and Equateur provinces [7, 8] (Supplementary Figure 1).

Both Salmonella Enteritidis and Typhimurium serotypes were equally present over the years 2011–2014, but local fluctuations occurred, in line with observations elsewhere in sub-Saharan Africa [21, 22]. The high proportion of Salmonella Enteritidis in the present study contrasted with the 18.0% proportion in the previous period [10]. Association with the rainy season was confirmed, particularly for Bas-Congo [10].

The present age and sex pattern of iNTS infection confirmed previously described patterns: Salmonella Typhi was not exclusively confined to school-aged children and adults but also affected children aged <5 years (20.3% of isolates vs 10.9% during the previous period) [9]. Invasive NTS affected younger children (>80% of isolates recovered in children <5 years) and was sporadically observed in neonates. However, in contrast with countries with higher HIV prevalence, no second age peak among adults (representing HIV infection) was noted [21], which can be partially explained by the relatively low incidence rate of HIV among adults in DRC. The male-to-female ratio for iNTS (1.2) was identical to what was observed in the 2007–2010 period [10].

Compared to the previous surveillance period (2007–2010), Salmonella Typhi showed a clear tendency to increased resistance, in particular for DCS, which was 37.2% currently vs 15.4% previously [9]; likewise, combined multidrug resistance and DCS occurred in 18.3% of isolates presently vs 7.5% previously [9]. In line with the findings of the previous period [10], DCS was invariably associated with mutations in the gyrA gene mostly at residue Ser83 and also at codon Asp87; plasmid-encoded mechanisms were rare. For iNTS, overall rates of multidrug resistance were similar (although slightly increased in Salmonella Typhimurium), and overall DCS rates were <2.5%. By contrast, combined azithromycin resistance and Ambler class A ESBL production was presently observed in >10% of Salmonella Typhimurium isolates, compared with rates <2% for either ESBL production or azithromycin resistance previously [10]. Unlike the previous period (during which only 3 ESBL-producing isolates with ESBL [SHV-2a] were found), ESBL production was shown in >10% of Salmonella Typhimurium isolates. They were presently geographically confined and all showed presence of the blaTEM-1 gene, suggesting close relatedness. In addition, most were co-resistant to azithromycin, which has not been reported before in sub-Saharan Africa. Acquisition of ESBL by INTS does not occur easily but threatens efficient treatment [23], and the spread of iNTS has been shown previously to be fueled by multidrug resistance [22]. As a consequence, the finding of these combined ESBL-producing/azithromycin-resistant Salmonella Typhimurium isolates in association with the apparent increase in iNTS cases in Bas-Congo is of high concern.

In conclusion, invasive salmonellosis represented the majority of CSOs causing BSI in DRC. Compared to data from the period 2007–2010, antibiotic resistance rates have risen considerably, which calls for sustained surveillance and appropriate public health measures to contain their spread and transmission.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Edmonde Bonebe, Brigitte Mapendo, Christian Balamika Pululu, Aimée Bitenga Luyindula, Joel Mifundu Nzuzi, Marleen Verlinden, Lara Balcaen, Kim van Bambost, and Bart Kleine Deters for technical support.

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. This study was supported by grant OPP1125993 of the Bill & Melinda Gates Foundation and the Belgian Directorate of Development Cooperation (DG) through Project 2.01 of the Third Framework Agreement between the Belgian DGD (Ministry of Development Cooperation) and the Institute of Tropical Medicine (ITM), Belgium. The department of pediatrics and the laboratory of the University Hospital of Kisangani are sponsored by the collaboration with KU Leuven in the project ZRDC2009EIN6 of the Flemish Interuniversity Council (VLIR-UOS),
funded by the Belgian DGD. L. M. K. has a scholarship from ITM supported
by the Belgian DGD. M.-F. P. has a scholarship from the Foundation Marguerite-Marie Delacroix, Tienen, Belgium. D. F. is supported by a DGD scholarship through the Royal Belgian Institute of Natural Sciences.

**Supplement sponsorship.** This article appeared as part of the supplement “Invasive Salmonellosis Disease in Africa,” sponsored by the University of Otago.

**Potential conflicts of interest.** All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


