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The genomic epidemiology of multi-drug resistant invasive non-typhoidal Salmonella in selected sub-Saharan African countries

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ABSTRACT

Background Invasive non-typhoidal Salmonella (INTS) is one of the leading causes of bacteraemia in sub-Saharan Africa. We aimed to provide a better understanding of the genetic characteristics and transmission patterns associated with multi-drug resistant (MDR) INTS serovars across the continent.

Methods A total of 166 INTS isolates collected from a multi-centre surveillance in 10 African countries (2010–2014) and a fever study in Ghana (2007–2009) were genome sequenced to investigate the geographical distribution, antimicrobial genetic determinants and population structure of INTS serotypes–genotypes. Phylogenetic analyses were conducted in the context of the existing genomic frameworks for various INTS serovars. Population-based incidence of MDR-INTS disease was estimated in each study site.

Results Salmonella Typhimurium sequence-type (ST) 313 and Salmonella Enteritidis ST11 were predominant, and both exhibited high frequencies of MDR. Salmonella Dublin ST10 was identified in West Africa only. Mutations in the gyrA gene (fluoroquinolone resistance) were identified in S. Enteritidis and S. Typhimurium in Ghana; an ST313 isolate carrying blactx-M-15 was found in Kenya. International transmission of MDR ST313 (lineage II) and MDR ST11 (West African clade) was observed between Ghana and neighbouring West African countries. The incidence of MDR-INTS disease exceeded 100/100 000 person-years-of-observation in children aged <5 years in several West African countries.

Key questions

What is already known?

► Invasive non-typhoidal Salmonella (INTS) disease is an emerging pathogen in sub-Saharan Africa.
► INTS is now a leading cause of bacteraemia in sub-Saharan Africa.
► The disease is associated with specific sequence types of S. Enteritidis and S. Typhimurium.

Conclusions We identified the circulation of multiple MDR INTS serovar STs in the sampled sub-Saharan African countries. Investment in the development and deployment of INTS vaccines coupled with intensified antimicrobial resistance surveillance are essential to limit the impact of these pathogens in Africa.

BACKGROUND

The non-typhoidal members of Salmonella enterica are archetypal zoonotic pathogens typically associated with self-limiting diarrhoea in humans. However, certain non-typhoidal Salmonella serovars are also a recognised cause of invasive disease in specific geographical regions. Invasive non-typhoidal Salmonella (iNTS) is...
What are the new findings?

- iNTS disease is widespread across the sampled locations.
- Salmonella Typhimurium sequence-type (ST) 313 and Salmonella Enteritidis ST11 are the predominant pathogens.
- There is a high frequency of multi-drug resistant (MDR) phenotypes.
- The incidence of MDR-intestinal typhus (iNTS) disease exceeded 100/100,000 person-years-of-observation in children aged <5 years in several West African countries.

What do the new findings imply?

- Sustained disease surveillance of MDR-iNTS organisms is essential.
- There is a need for acceleration of iNTS vaccines.
- We need a better understanding of the disease reservoir and human-to-human transmission.

METHODS

Ethics approval and consent to participate

This research was conducted under the ethical principles of the Declaration of Helsinki. The IVI Institutional Review Board (IRB), the national ethical review committees in each participating country, and local research ethics committees approved the study protocol. All eligible patients meeting the study inclusion criteria were provided with a detailed explanation of the study purpose, and written informed consent was obtained prior to study enrolment. For children, written informed consent was obtained from parent or guardian.

Study design and inclusion criteria

The majority of iNTS isolates (117/166) in this study originated from the Typhoid Fever Surveillance in Africa Program (TSAP), conducted in 13 sites in 10 countries between 2010 and 2014. Febrile patients from all age groups (except in Ghana, where only children aged <15 years were enrolled) with a tympanic or axillary temperature of ≥38.0°C or ≥37.5°C, respectively, living in a defined study catchment area were eligible for recruitment. For inpatients, reported fever within 72 hours of admission was also necessary for inclusion. Written informed consent/assent was obtained. Clinical assessments of patients included history of illness, physical examination and clinical appraisal. Blood samples (5–10 mL for adults; 1–3 mL for children) were collected for microbiological testing and diagnosis. An additional 49 iNTS isolates were obtained from a febrile surveillance study conducted at the Presbyterian Hospital of Agogo in Ghana, between 2007 and 2009.

Patient and public involvement statement

This TSAP study was performed under a single protocol with some site-specific and country-specific details; these details were developed with the study sites and local patient groups and communities attending the healthcare facilities. These data were essential for establishing the demographic framework of the sites and understanding how patients accessed healthcare and disease diagnosis. Therefore, these patient/community groups...
Table 1  Distribution of iNTS serovars and genotypes circulating in the sampled countries in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Country (number of iNTS*)</th>
<th>Serovars</th>
<th>Number of iNTS per serovar (b)</th>
<th>% of total number of iNTS per country (b)/(a)</th>
<th>Genotype (sequence type)</th>
<th>Number of iNTS per genotype (c)</th>
<th>% of total number of iNTS per country (c)/(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkina Faso (12) Typhimurium</td>
<td>7</td>
<td>58</td>
<td></td>
<td>313</td>
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<tr>
<td></td>
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<td>33</td>
<td>11</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
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<td>8</td>
<td>10</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Ghana (133) Typhimurium</td>
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<td></td>
<td>313</td>
<td>90</td>
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</tr>
<tr>
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<td>11</td>
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<tr>
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<td>13</td>
<td>10</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
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<tr>
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<td>1</td>
<td>308</td>
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<td>359</td>
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<td>145</td>
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<td>313</td>
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<td>Madagascar (4) Typhimurium</td>
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<td>11</td>
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<td>ST11</td>
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<tr>
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<td>ST10</td>
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<td>11</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>8</td>
<td>5</td>
<td>Other STs§</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*iNTS: invasive non-typhoidal Salmonella.
†Total 8 countries: Burkina Faso, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, South Africa and Tanzania presented in this table. No iNTS isolates were yielded in Sudan and Ethiopia.
‡Other sequence types of S. Enteritidis detected: 1 ST183 (isolate yielded from Ghana; age/sex unknown due to missing data) and 1 ST2107 (from Ghana; a 22-year-old woman); both non-MDR and no antimicrobial resistant genes detected.
§Sequence types of the other NTS serovars: 1 Muenster ST321 (yielded from Ghana; age/sex/year unknown due to missing data), 1 Poona ST308 (yielded from Ghana in 2008; age/sex unknown due to missing data), 1 Stanleyville ST339 (yielded from Ghana; age/sex/year unknown due to missing data), 1 Virchow ST359 (from Ghana; age/sex/year unknown due to missing data), 3 Choleraesuis ST145 (two isolates yielded from 1-year-old female infants in 2010 and 2011 and 1 from a 3-year-old female infant in 2012; all from Guinea-Bissau), 1 unknown ST2533 from Tanzania.
inform the study design to address issues regarding healthcare access and practices. Local communities and patients acted as communicators in the local community to encourage people to attend healthcare facilities when symptomatic. Data from the studies have been provided to study sites to inform the local community and patients of the study findings.

Bacterial isolates and antimicrobial susceptibility testing

Blood specimens were inoculated into an aerobic blood culture bottle and incubated in systems with automated growth detection (BACTEC Peds Plus Medium/BACTECT Plus Aerobic-F, BACTEC, Becton-Dickinson, New Jersey; or BacT/ALERT PF Paediatric FAN/BacT/ALERT FA FAN Aerobic, bioMerieux, Marcy l’Etoile, France). Blood cultures with bacterial growth were sub cultured on blood and chocolate agar (Oxoid, Basing stoke, UK), and biochemical tests were conducted (API 20E; bioMerieux) to identify suspected Salmonella isolates. Antimicrobial susceptibility testing was performed using agar diffusion tests according to the Clinical Laboratory and Standards Institute guidelines.

Data sources and bacterial isolates

A total of 166 iNTS isolates were used for this investigation, which comprised 94 iNTS isolates from the TSAP study, an additional 23 iNTS isolates obtained from outside predefined TSAP study catchment areas and 49 iNTS isolates from other studies. In order to place these S. Typhimurium and S. Enteritidis isolates into a global phylogenetic context, the existing datasets were incorporated: 147 iNTS serotype Typhimurium ST313 isolates from seven countries (Malawi, Kenya, Mozambique, Uganda, DRC, Nigeria and Mali); 11 Nigeria and DRC; 12 Malawi; 16 Kenya; 14 Malawi and 594 iNTS serotype Enteritidis ST11 isolates (selected from Feasey et al.; online supplemental table 1).

Whole genome sequencing

Genomic DNA was extracted from all Salmonella isolates using the Wizard Genomic DNA Extraction Kit (Promega, Wisconsin, USA). Two micrograms of genomic DNA from each organism was subjected to indexed-tagged pair-end sequencing on an Illumina HiSeq 2000 platform (Illumina, CA, USA) at the Wellcome Sanger Institute to generate 100 bp paired-end reads. Data quality control was performed using in-house pipelines. Raw sequence data are available in the European Nucleotide Archive (Project number: ERP009684, ERP010763, ERP013866) (online supplemental table 2).

Single nucleotide polymorphism (SNP) calling and analyses

Raw Illumina reads were used to create multiple assemblies using Velvet V.1.2.35 with parameters optimised using VelvetOptimiser V.2.2.5.45 and automated annotation was performed using PROKKA V.1.5.36 Roary37 was used to define the pan genome of 166 iNTS isolates with blastp percentage identity of 99% and a core definition of 99%. In total, 3450 core genes were identified (genes that were present in ≥99% strains) and 86765 SNP sites were extracted from the core gene alignment using SNP-sites V.2.1.3.35

For S. Typhimurium ST313, raw Illumina reads of 99 isolates from this study and 147 S. Typhimurium ST313 from previous studies11 12 29 33 38 were mapped to the reference sequence of S. Typhimurium strain SL1344 (accession: FQ312003.1), using SMALT V.0.7.4 (http://www.sanger.ac.uk/resources/software/smalt/). Candidate SNPs were called against the reference sequence using SAMtools38 and filtered with a minimum mapping quality of 30 and minimum consensus base agreement of 75%. The allele at each locus in each isolate was determined by reference to the consensus base in that genome using SAMtools mpileup and removing low confidence alleles with consensus base quality ≤20, read depth ≤5 or a heterozygous base call. The repeat finding program in NuCmer V.3.1.39 was used to identify exact repetitive regions of ≥20 bp in length in the reference genome and SNPs called in these regions were excluded. SNPs called in phage sequences and recombinant regions identified using Gubbins40 were further removed, resulting in a final set of 1960 chromosomal SNPs. The identification of SNPs for S. Enteritidis ST11 was performed following the same procedure as S. Typhimurium ST313. Briefly, the raw Illumina reads of 28 S. Enteritidis ST11 isolates from this study and 594 additional isolates from a global collection13 were mapped to the reference sequence of S. Enteritidis strain P125109 (accession: NC_011294.1), using SMALT.
followed by SNP calling and filtering as described previously, resulting in a final set of 25,121 SNPs.

Phylogenetic analyses

A maximum likelihood (ML) phylogenetic tree was constructed from the 86,765 SNP alignment of all 166 iNTS isolates using RAxML V.8.2.8 with a generalised time-reversible model and a Gamma distribution to model the site-specific rate variation (GTRGAMMA). Clade support for this tree was assessed through a bootstrap analysis with 100 pseudo-replicates. To investigate the molecular epidemiology of the S. Typhimurium ST313 and S. Enteritidis ST11 isolates sequenced here in a global context, a ML tree was inferred from an alignment of 1960 SNPs for 246 S. Typhimurium ST313 (99 from this study and 147 from previous studies) and an alignment of 25,121 SNPs for 622 S. Enteritidis ST11 isolates (28 from this study and 594 from a global collection), using RAxML with GTRGAMMA model. Support for these phylogenetic trees was assessed through a 100 bootstrap pseudo-analysis. Tree annotation was visualised using ITOL.

Antimicrobial resistance gene and plasmid analyses

From raw Illumina reads, Short Read Sequence Typing—SRST2 was used to identify acquired AMR genes and their precise alleles using the ARG-annot database, as well as plasmid replicons using the PlasmidFinder database. Multi-locus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replications using the PlasmidFinder database. Multilocus sequence typing (MLST) of all iNTS isolates was also determined using SRST2 together with the MLST database for Salmonella enterica downloaded from pubMLST (https://pubmlst.org/organisms/salmonella-spp). Salmonella serotypes were identified using conventional serology as well as plasmid replication...
incidence of S. Typhi and iNTS disease in the TSAP study was used to calculate MDR iNTS incidence. 7 30 31 Demographic data from Health and Demographic Surveillance System (HDSS) sites were used to estimate population denominators where available. In non-HDSS sites, health-seeking behaviour as reported by randomly administered healthcare utilisation surveys were used to estimate population denominators for each study site. Crude incidence rates were adjusted to account for the proportion of surveyed individuals who reported seeking care for a febrile episode at a study facility to yield adjusted incidence rates. Adjusted incidence rates of MDR iNTS cases per 100 000 per person-years-of-observation (PYO) were estimated with 95% CIs using these adjustment factors and crude MDR iNTS case numbers. The previously established multi-country database (Microsoft Visual FoxPro 7.0, Redmond, Washington) for TSAP was used for the countries with MDR iNTS isolates.

RESULTS
Geographical distribution of iNTS serotypes and sequence types
The majority (66%; 110/166) of iNTS organisms in the sampled sub-Saharan African countries were S. Typhimurium, of which 90% (99/110) were ST313 and 10% (11/110) were ST19. S. Enteritidis accounted for 18% (30/166) of the isolates, composed of ST11 (93.3%; 28/30), ST183 (3.3%; 1/30) and ST2107 (3.3%; 1/30). S. Dublin (ST10) comprised a further 11% (18/166) of isolates. Several other serotype STs, including S. Choleraesuis ST145 (3/166), S. Muenster ST321 (1/166), S. Poona ST308 (1/166), S. Stanleyville ST339 (1/166) and S. Virchow ST359 (1/166), were also identified. S. Typhimurium ST313 was mostly limited to West Africa (table 1), whereas S. Enteritidis ST11 appeared to be pervasive in both West and Southern Africa. S. Dublin ST10 was identified in West Africa and S. Typhimurium ST19 was distributed across the continent.

Overall, 61% (102/166) of the iNTS organisms described here were MDR. These were isolated in Burkina Faso (83%; 10/12), Ghana (66%, 88/133), Guinea-Bissau (22%; 2/9), Kenya (100%; 1/1) and Senegal (50%; 1/2). S. Typhimurium exhibited the highest prevalence of MDR (85%; 94/110); 95% (94/99) of the ST313 isolates were MDR. In total, 23% (7/30) of S. Enteritidis and 6% (1/18) S. Dublin organisms were MDR; none of the S. Typhimurium ST19 were MDR (figure 1, table 2).

Phylogenetics and AMR of iNTS
A phylogenetic reconstruction of all iNTS isolates showed that the three major serovar STs—S. Typhimurium (ST313), S. Enteritidis (ST11) and S. Dublin (ST10)—formed independent clusters with dissimilar AMR gene profiles (figure 2). Almost all of the MDR S. Typhimurium ST313 (95%; 94/99) carried the Tn21 transposon-associated MDR-loci (sulII-strAB-dfrA1-aadA1-sulI-cat-blaTEM) on an IncF virulence-resistance plasmid (pSLT-BT). 11 A single MDR S. Typhimurium ST313 from Kenya additionally carried two copies of blaCTX-M-1, conferring resistance to third-generation cephalosporins. One copy of blaCTX-M-1 was located on the 300 kb IncHI2 plasmid, pKST313 (accession number: LN794248), and
Reduced susceptibility to fluoroquinolones in *S.* Typhimurium ST313 was uncommon, with 2% (2/99) of Ghanaian ST313 isolates possessing a single mutation (S87Y) in *gyrA* (table 2). The majority of *S.* Enteritidis ST11 (19/28; 68%) harboured the typical IncF virulence plasmid (pSENV, accession number NC_019120.1, coverage 100%, identity 99%). The remaining *S.* Enteritidis ST11 (9/28; 32%) harboured a novel IncI1 virulence-resistance plasmid (pSEP, accession number: ERP121368) of approximately 68 kb (figure 3), of which 7/28 (25%) isolates (4 from Burkina Faso, 2 from Ghana, 1 from Senegal) carried the MDR-encoding Tn21-like transposon (*sulII-strAB-dfrA1-aadA1-sulI-cat-blaTEM*), and 2/28 (7.1%) isolates carried a different AMR cassette (*TnpA-sulII-strAB-blaTEM-Tn3*). The novel IncI plasmid exhibited 60% homology to pSENV but did not harbour the IncF replicon, the *pefBACD* fimbriae-encoding operon, or the virulence-associated genes *srgA* and *rck* (figure 3). In addition, two non-MDR Ghanaian *S.* Enteritidis ST11 possessed an AMR cassette (*sul2-strAB-aadA1-tetA*) carried on a small (11 kb) non-conjugative IncQ plasmid conferring resistance against sulfonamides, streptomycin and tetracyclines. This IncQ plasmid exhibited a similar genetic structure to pSTU288-2 from *S.* Typhimurium (accession number CP004059.1, coverage 98%, identity 99%). Two further non-MDR Ghanaian isolates carried a Tn21-mediated AMR cassette (*sulII-strAB-dfrA1-aadA1-sulI-cat-blaTEM*) on the virulence plasmid, and a single non-MDR isolate from Madagascar carried a *bla*~TEM~ Tn3 integrated into the virulence plasmid. Reduced susceptibility to fluoroquinolones was predicted by the sequences in 39% (11/28) of the *S.* Enteritidis-ST11, all of which originated from Ghana and displayed a variety of *gyrA* mutations (D87G: 6 isolates, D87N: 3 isolates, D87Y: 2 isolates) (table 2).

**Phylogenetics of *S.* Typhimurium ST313 and *S.* Enteritidis ST11 isolates in global context**

To investigate the population structures of *S.* Typhimurium ST313 and *S.* Enteritidis ST11 in a broader context, we constructed global phylogenies. All *S.* Typhimurium ST313 isolates from our study fell into lineage II (figure 4A). The single Kenyan ST313 isolate carrying two copies of *blaCTX-M-15* was part of the previously described clonal expansion of MDR ceftriaxone-resistant ST313 sub-lineage. Notably, the Ghanaian ST313 isolates did not form a single cluster, but were associated with multiple clusters from Mali, Burkina Faso and Nigeria, indicating multiple introduction events (figure 4A).

A detailed phylogenetic investigation of *S.* Enteritidis ST11 demonstrated that 11/28 (40%) (Ghana: 5, Burkina Faso: 4, Senegal: 1, Guinea Bissau: 1) isolates fell into the...
West African clade. The ST11 organisms within this West African clade displayed either MDR (seven isolates) or other non-MDR AMR phenotypes (two isolates). We found evidence that some Ghanaian isolates within this clade clustered alongside organisms from Burkina Faso and Mali, again suggesting international transmission. In addition, 13/28 (46%) of the S. Enteritidis ST11 (Ghana: 11, Madagascar: 1, South Africa: 1) belonged to the Global epidemic clade. These isolates had phylogenetic links with their country-specific clusters, with the exception of two Ghanaian isolates that clustered with organisms from neighbouring Cameroon and Senegal. Lastly, 4/28 (14%) (2 from Ghana, 2 from Madagascar) of the ST11 isolates grouped within the outlier cluster (figure 4B).

Figure 4  Phylogenetics of S. Typhimurium ST313 lineage II and S. Enteritidis ST11 in sub-Saharan Africa. (A) Maximum likelihood phylogenetic tree of S. Typhimurium-ST313 lineage II isolates from this study in the context of the sub-Saharan African continent; lineage I was pruned to enhance the visualisation of the tree. Red circles at the terminal leaves correspond to our study isolates. The first column shows different colour-coded countries from where all analysed isolates originate. The second column shows MDR and non-MDR isolates in red and grey, respectively. (B) Maximum likelihood phylogenetic tree of our S. Enteritidis-ST11 isolates in the global context of S. Enteritidis. Red circles at the terminal leaves correspond to our study isolates. The first and second columns show regions and resistant phenotypes in different colours. The tree scale bar indicates the number of substitutions per variable site.

Incidence of MDR iNTS disease in sub-Saharan Africa

We calculated the age-stratified incidence rates of MDR iNTS in previously described study catchment areas in Burkina Faso, Ghana, Guinea-Bissau and Kenya (table 3). The adjusted incidence of MDR iNTS disease exceeded 100/100 000 PYO in children <15 years of age in all West African countries: Burkina Faso (Nioko II, 274/100 000 PYO, 95% CI 185 to 406; Polesgo, 255/100 000 PYO, 95% CI 138 to 470), Ghana (Asante Akim North: Ghana-AAN, 414/100 000, 95% CI 333 to 515) and Guinea-Bissau (Bandim, 105/100 000, 95% CI 69 to 161). Among children <15 years, younger children (<2–4 years) exhibited the highest MDR iNTS incidence rates in both sites in Burkina Faso: 753/100 000 PYO (95% CI 460 to 1233) in Nioko II and 630/100 000 PYO (95% CI 288 to 1380) in Polesgo. In both settings in Burkina Faso, the incidence of MDR iNTS disease in the infant age group was slightly lower than in the group aged 2–4 years, but children <5 years old exhibited a high burden of MDR iNTS disease. In Ghana-AAN, infants aged <2 years had the highest incidence of MDR iNTS disease (1435/100 000; 95% CI 1110 to 1854) followed by children aged between 2 and 5 years (747/100 000; 95% CI 491 to 1135). Similarly, in Guinea-Bissau, infants <2 years old exhibited the highest incidence of MDR iNTS disease (291/100 000; 95% CI 176 to 482). The incidence rate of MDR iNTS in older age groups (≥15 years) was relatively...
### Table 3  Incidence estimates of MDR iNTS disease in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Country*</th>
<th>Age group in years</th>
<th>PYO estimation†</th>
<th>Proportion of catchment population visiting study facility in case of fever (95% CI)</th>
<th>Catchment population</th>
<th>Catchment population adjusted by health-seeking behaviour</th>
<th>PYO estimation†</th>
<th>Geno-sequence iNTS cases</th>
<th>Crude MDR iNTS cases</th>
<th>Crude MDR iNTS incidence per 100 000 PYO</th>
<th>Adjusted MDR iNTS cases</th>
<th>Adjusted MDR iNTS incidence per 100 000 PYO (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Burkina Faso</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nioko II</td>
<td>0–1</td>
<td>81% (74 to 88)</td>
<td>2200</td>
<td>1788</td>
<td>2097</td>
<td>247/1297 (19)</td>
<td>2</td>
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<td>48</td>
<td>5</td>
<td>251 (107 to 590)</td>
</tr>
<tr>
<td></td>
<td>2–4</td>
<td>81% (75 to 86)</td>
<td>1823</td>
<td>1477</td>
<td>2097</td>
<td>235/1259 (19)</td>
<td>3</td>
<td>3</td>
<td>143</td>
<td>16</td>
<td>753 (460 to 1233)</td>
</tr>
<tr>
<td></td>
<td>5–14</td>
<td>81% (78 to 84)</td>
<td>4295</td>
<td>3479</td>
<td>4889</td>
<td>228/889 (26)</td>
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<td>1</td>
<td>20</td>
<td>4</td>
<td>79 (29 to 214)</td>
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<tr>
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<td>7</td>
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<td>55</td>
<td>25</td>
<td>274 (185 to 406)</td>
</tr>
<tr>
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<td>≥15</td>
<td>81% (79 to 83)</td>
<td>9428</td>
<td>7637</td>
<td>10 676</td>
<td>208/759 (27)</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>35 (13 to 96)</td>
</tr>
<tr>
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<td>14 381</td>
<td>19 759</td>
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<td>8</td>
<td>6</td>
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<td>29</td>
<td>145 (100 to 290)</td>
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<td>896</td>
<td>824</td>
<td>929</td>
<td>117/475 (25)</td>
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<td>1</td>
<td>108</td>
<td>4</td>
<td>431 (162 to 1147)</td>
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<tr>
<td></td>
<td>2–4</td>
<td>83% (76 to 89)</td>
<td>856</td>
<td>710</td>
<td>992</td>
<td>148/466 (32)</td>
<td>2</td>
<td>2</td>
<td>202</td>
<td>6</td>
<td>630 (288 to 1380)</td>
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<td>87% (83 to 91)</td>
<td>1734</td>
<td>1509</td>
<td>2104</td>
<td>252/510 (49)</td>
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<td>3557</td>
<td>4917</td>
<td>239/629 (38)</td>
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<td>1</td>
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<td>3</td>
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<td>13</td>
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<tr>
<td>AAN</td>
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<td>11 222</td>
<td>1760</td>
<td>4080</td>
<td>41</td>
<td>88</td>
<td>24</td>
<td>588</td>
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<td>1435 (1110 to 1854)</td>
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<td>8086</td>
<td>1268</td>
<td>2940</td>
<td>41</td>
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<td>306</td>
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<td>5415</td>
<td>12 554</td>
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<td>147</td>
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<td>n.a.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bandim</td>
<td>0–1</td>
<td>46% (39 to 54)</td>
<td>10 852</td>
<td>4992</td>
<td>5198</td>
<td>206/631 (33)</td>
<td>7</td>
<td>2</td>
<td>96</td>
<td>15</td>
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<td>19 905</td>
<td>8360</td>
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<td>20 165</td>
<td>n.a.</td>
<td>9</td>
<td>2</td>
<td>40</td>
<td>21</td>
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</tr>
<tr>
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<td>≥15</td>
<td>45% (43 to 47)</td>
<td>62 694</td>
<td>28 212</td>
<td>37 109</td>
<td>105/163 (64%)</td>
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<td>57 274</td>
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<td>2</td>
<td>14</td>
<td>21</td>
<td>37 (24 to 57)</td>
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<td><strong>Kenya</strong></td>
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<td>3467</td>
<td>1456</td>
<td>2031</td>
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<tr>
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<td>2–4</td>
<td>39% (36 to 43)</td>
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<td>1197</td>
<td>2039</td>
<td>312/312 (100)</td>
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</table>

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<table>
<thead>
<tr>
<th>Country</th>
<th>Age group in years</th>
<th>Proportion of catchment population visiting study facility in case of fever (95% CI)</th>
<th>Catchment population adjusted by health-seeking behaviour</th>
<th>PYO</th>
<th>Recruitment proportion†</th>
<th>Genome-sequenced iNTS cases</th>
<th>Crude MDR iNTS cases</th>
<th>Crude MDR iNTS incidence per 100 000 PYO</th>
<th>Adjusted MDR iNTS cases</th>
<th>Adjusted MDR iNTS incidence per 100 000 PYO (95% CI)‡</th>
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<td>Senegal</td>
<td>5–14</td>
<td>43% (39 to 47)</td>
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<td>539/539 (100)</td>
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<td>≥15</td>
<td>35% (32 to 38)</td>
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<td>5342</td>
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<td>301/301 (100)</td>
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<td>1</td>
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<tr>
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<td>2–4</td>
<td>37% (33 to 41)</td>
<td>30 180</td>
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<tr>
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<td>5–14</td>
<td>31% (28 to 34)</td>
<td>96 152</td>
<td>29 807</td>
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<td>1</td>
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</tr>
<tr>
<td></td>
<td>≥15</td>
<td>30% (28 to 31)</td>
<td>195 726</td>
<td>58 718</td>
<td>83 874</td>
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<td>1</td>
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<tr>
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<td>342 178</td>
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<td>2</td>
<td>1</td>
<td>n.a</td>
<td>n.a</td>
</tr>
</tbody>
</table>

*TSAP was performed in 10 countries, of which 8 countries (Burkina Faso, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, South Africa and Tanzania in alphabetical order) exhibited positive iNTS isolates confirmed via whole genome sequencing. MDR iNTS were found in 5/8 countries presented in this table. No MDR iNTS were detected via whole genome sequencing from the isolates yielded from Madagascar, South Africa and Tanzania.
†PYO estimation methodologies have been published in detail in the TSAP typhoid burden paper (Marks et al, Lancet Global Health, 2017).
‡Adjusted MDR iNTS incidence per 100 000 PYO (95% CI); adjustments for case recruitment and error factors.
§Ghana samples included in this estimation of MDR iNTS are from the TSAP only.
¶Non-TSAP includes other fever surveillance performed in Agogo ("IsolateAgogo", "FISA" and "zTYSA"). Refer to the Methods section.
**Adjusted MDR incidence of iNTS per 100 000 PYO could not be estimated for the study site in Senegal due to unavailable data on the recruitment proportion. The one MDR iNTS patient confirmed and presented in this table (crude MDR iNTS case) was a male infant aged 17 months, infected in 2012 with S. Enteritidis ST11. The one non-MDR iNTS patient presented in this table (genome-sequenced iNTS case) was a male adult aged 66 years, infected in 2012 with S. Typhimurium. n.a., not available; PYO, person-years-of-observation.
Our study shows that MDR iNTS is highly prevalent in several sub-Saharan African countries. Specifically, we found that MDR S. Typhimurium ST313 is the most common cause of iNTS disease, but that other iNTS serovars, principally S. Enteritidis ST11 and S. Dublin ST10 in West Africa, also constitute a major proportion of the disease burden.52 53  Our phylogenetic analyses provide further evidence for the regional transmission of two MDR serovars/STs (S. Typhimurium ST313 and S. Enteritidis ST11) between Ghana and neighbouring countries Burkina Faso, Nigeria, Mali and Senegal. These transmission events highlight the need for intensified AMR surveillance, the coordination of AMR reporting, and sustained public health control measures between these and other African countries.

We calculated a particularly high incidence of MDR iNTS disease in the West African countries of Burkina Faso, Ghana (<5 years) and Guinea-Bissau (<2 years). The incidence rates low, ranging between 0 (Guinea-Bissau) to 11 (Kenya) and 54 (Burkina Faso) per 100 000 PYO.

**DISCUSSION**

Our study shows that MDR iNTS is highly prevalent in several sub-Saharan African countries. Specifically, we found that MDR S. Typhimurium ST313 is the most common cause of iNTS disease, but that other iNTS serovars, principally S. Enteritidis ST11 and S. Dublin ST10 in West Africa, also constitute a major proportion of the disease burden.52 53  Our phylogenetic analyses provide further evidence for the regional transmission of two MDR serovars/STs (S. Typhimurium ST313 and S. Enteritidis ST11) between Ghana and neighbouring countries Burkina Faso, Nigeria, Mali and Senegal. These transmission events highlight the need for intensified AMR surveillance, the coordination of AMR reporting, and sustained public health control measures between these and other African countries.

We calculated a particularly high incidence of MDR iNTS disease in the West African countries of Burkina Faso, Ghana (<5 years) and Guinea-Bissau (<2 years). The incidence rates low, ranging between 0 (Guinea-Bissau) to 11 (Kenya) and 54 (Burkina Faso) per 100 000 PYO.
of MDR iNTS disease presented here generally correspond with the incidence of iNTS disease in other African countries.\textsuperscript{7} Effective antimicrobial therapy is an essential component of iNTS management; however, the effectiveness of first-line treatments has been diminished due to the emergence and spread of MDR and XDR NTS strains.\textsuperscript{13-29,54} Our data also depict the emergence of reduced fluoroquinolones susceptibility in both MDR ST313 and ST11 in Ghana, as well as the circulation of a ceftriaxone-resistant ST313 sub-lineage in Kenya. Invasive \textit{Salmonella} with reduced susceptibility to ciprofloxacin have been reported in Burkina Faso,\textsuperscript{7} Ghana,\textsuperscript{2,721} Nigeria,\textsuperscript{6,16} Senegal,\textsuperscript{16} Mozambique,\textsuperscript{28,56} the Congo,\textsuperscript{28,56} Kenya\textsuperscript{28} and South Africa.\textsuperscript{28} This increasing trend in resistance against clinically important classes of antimicrobials in differing iNTS serovars across Africa is of major concern.\textsuperscript{14} The growing use of ceftriaxone and ciprofloxacin for the treatment of febrile illnesses in Africa may lead to an increase in of MDR and XDR pathogens in this continent, which has already been observed across Asia in the last two decades.

Several limitations should be considered in interpreting and generalising these data beyond our study sites. While we are able to illustrate the magnitude of the problem of MDR iNTS disease in West Africa, there are relatively few genomic data points available from countries in East and Southern Africa. Previous studies showed high prevalence of MDR iNTS disease in Kenya\textsuperscript{27,58} and a recent meta-analysis suggested that MDR iNTS has emerged across four regions of sub-Saharan Africa.\textsuperscript{60} The generation and analysis of additional epidemiological and genomic iNTS data in Eastern/Southern Africa would help facilitate comparison of incidence rates and AMR profiles of iNTS-associated organisms between African regions. Further, the estimated incidence rates of MDR iNTS disease in our study should be interpreted with caution as the number of cases in some countries were relatively small. In parallel, the original study design may also have led to some underestimation of iNTS burden, as afebrile patients with other clinical symptoms associated with iNTS disease were not enrolled. As a result, the true incidence of MDR iNTS disease in some settings need to be further monitored with more systematic disease surveillance.

Despite the identified limitations, our study provides enhanced insights into the population structure and transmission dynamics of major MDR iNTS serovars in sub-Saharan Africa and identified countries with a high burden of MDR iNTS. There is an urgent need to expand clinical and genomic surveillance for pathogens causing bloodstream infections across continental Africa to improve our understanding of disease incidence and to monitor AMR trends. Such data can better inform antimicrobial stewardship to extend the life of existing antimicrobial therapies and prioritisation of preventative interventions including vaccines. The development and deployment of a safe, low-cost, highly efficacious multivalent vaccine should be prioritised for the management and prevention of iNTS disease in Africa, particularly in countries with high prevalence of MDR iNTS infections, as well as HIV, malaria and malnutrition.\textsuperscript{61-62} Meanwhile, further investigations of household transmission dynamics and human and non-human reservoirs of infection are warranted to inform better iNTS control measures and, ultimately, optimal programmatic use of future vaccines.

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