Molecular And Phenotypic Characterisation Of Non-Typhoidal \textit{Salmonella enterica} Associated With Human Disease In The Gambia

Thesis

How to cite:

For guidance on citations see FAQs.

© 2021 Saffiatou Darboe

https://creativecommons.org/licenses/by-nc-nd/4.0/

Version: Version of Record

Link(s) to article on publisher’s website:
http://dx.doi.org/doi:10.21954/ou.ro.00014a4a

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online’s data policy on reuse of materials please consult the policies page.

oro.open.ac.uk
Molecular and Phenotypic Characterisation of non-Typhoidal Salmonella enterica Associated with Human Disease in The Gambia.

Saffiatou Darboe- BSc (Hons), MSc
F4501154

Date of submission: 30th September 2021

Thesis submitted to the Open University in fulfilment of the requirement for the degree of Master of Philosophy (MPhil) in the School of Life, Health and Chemical Sciences.

In collaboration with Affiliated Research Centre: Medical Research Council Unit, The Gambia at the London School of Hygiene and Tropical Medicine (MRCG@LSHTM). P.O Box 273. Banjul. The Gambia, West Africa.

Director of Studies: Dr. Brenda Kwambana-Adams PhD; University College London, UK
Academic Collaborator: Dr. Richard Bradbury PhD; Federation University, Australia
Internal Supervisor: Prof Martin Antonio PhD; MRCG@LSHTM, The Gambia
External Supervisor: Prof Samuel Karinski PhD; Kenya Medical Research Council, Kenya
ABSTRACT

Background: Non-typhoidal Salmonella (NTS) are a major cause of foodborne gastroenteritis worldwide but have emerged as a significant cause of multi-drug resistant invasive disease in sub-Saharan Africa (sSA). Specific lineages of the Salmonella enterica subspecies enterica serovar Enteritidis (S. Enteritidis) and Salmonella enterica subspecies enterica serovar Typhimurium (S. Typhimurium) serovars are implicated, and genomic analysis has shown that these lineages in sSA are phylogenetically distinct from others causing gastroenteritis globally.

Methods: One hundred NTS isolates were collected from 93 patients presenting with clinical disease in 2001 from the eastern and 2006 to 2018 in the western regions of The Gambia. The isolates from the western region (n=80) were from blood (48), stool (25), cerebrospinal fluid (CSF; 1), abscesses (5), and urine (1) while those from the eastern region (n=20) also originated from blood (17), 2 CSF (2) and stool (1). The isolates were characterised by whole genome sequencing using Illumina platforms. Phenotypic susceptibility testing was done using Kirby Bauer disk diffusion. The sequence reads were analysed to determine lineages. Phylogenetic analysis was performed in the context of other African isolates from the European Nucleotide Archive.

Results: A total of 93 isolates (64 invasive, 23 gastroenteritis and 6 other infections) representing a single infection episode was analysed. S. Typhimurium (26/64; 30.6%) and S. Enteritidis (13/64; 20.3%) were the leading serovars associated with invasive disease respectively; whilst other serovars were mainly responsible for gastroenteritis (17/23; 73.9%). The presence of three major S. Enteritidis clades including the invasive West African clade (11/16; 68.8%) was confirmed. MDR was confirmed for seven isolates among the West African S. Enteritidis clade.

Conclusions: The study revealed insight into the dynamics of clinical NTS serovars in The Gambia. It has confirmed the presence of virulent multi-drug resistant S. Enteritidis highlighting the significance of surveillance in developing treatment guidelines.
ACKNOWLEDGEMENT

This thesis is the result of combined efforts of many people who made significant contributions to bring it to fruition.

First and foremost, I am fortunate to have been given this rare opportunity to embark on this life-changing journey by my wonderful supervision team. I am indebted to my internal Supervisor, Professor Martin Antonio and privileged to have Professor Samuel Kariuki, one of the best in the field as my external Supervisor! I am especially grateful for the financial support, mentorship, support, and advice during the project. I am humbled by the confidence you both have in me to bring this project to fruition and produce results. I am eternally grateful to you both.

I am particularly grateful to Dr. Brenda Kwambana-Adams, my Director of Studies for her relentless support and advice. You have been an incredible supervisor and an ‘awesome’ critique! Thank you for the encouragement, confidence, and steadfastness which has indeed improved my research skills.

I am forever indebted to my academic collaborator and career mentor Dr. Richard Bradbury. You ignited the flames and nurtured the seed of a great mind. I remain indebted to you in my scientific career as the pillar of my growth! Thank you for consistently believing in me and for the quick reviews.

I am immensely blessed to have the support of wonderful women who have taken a keen interest in my professional growth becoming mentors and research collaborators, Drs. Anna Roca, Effua Usuf and Uduak Okomo! Thank you for believing, motivating, and supporting me to bring my passion to fruition and the shoulders to lean on during challenging times. Uduak, thank you for the humour and ‘harassing’ me to multi-task!

Sincere gratitude and acknowledgement go to Dr. Blanca Perez-Sepulveda and Dr Luria Leslie Founou for proof-reading the thesis. I also thank Dr. Omai Garner, Dr. Abdul Karim Sesay, Dr. Jody Phelan and Mr Abdul Khalil Muhammed for their continued support throughout the project.

I am sincerely grateful to Dr. Davis Nwakanma for the support as I explored my research passion! Thank you for your confidence in trusting me to complete this task in addition to managing the Lab. Special gratitude to the one that introduced me to microbiology, Dr. Ousman Secka. Thank you for your mentorship and supervision!

To my colleagues in the Clinical labs, especially Buntung Ceesay and Mamadou Jallow you have been phenomenal!

Finally, I am grateful to my family for their understanding, support, and encouragement.

I now spread my wings to infinite possibilities!
DEDICATION

Dedicated to my mother, Ms Sarah Z. Nicolls who has always been my anchor.

To my children: Batch Samba Darboe, Nyima Saffie Darboe, Oumie Sarata Darboe and Tutty Isatou Darboe. Your love and support energised me!

To my patient husband, THANK YOU!!

Grateful to the Almighty God for health, abundance, and strength.

Scanning electron micrograph showing Salmonella invading human cells (Picture credit: Rocky Mountain Laboratories, NIAID, NIH)
STATEMENT OF WORK PERFORMED BY SUPERVISORS AND COLLEAGUES

❖ Professor Martin Antonio was the main supervisor of the MPhil study. He contributed to the design, guidance, and review of the articles. He was the senior investigator and provided support, mentorship, funding, and advice during the project.

❖ Dr. Brenda Kwambana-Adams was the director of my MPhil study. She contributed to the design of the study, review of manuscripts, providing guidance and mentorship.

❖ Dr. Richard S Bradbury was the academic collaborator who assisted in providing tutorial on research methodology and academic writing, contributed to the design of the study, and review of the articles.

❖ Dr. Samuel Kariuki was the external supervisor of the MPhil study contributing to the design, guidance, and review of the articles.

❖ Dr. Perez-Sepulveda was a collaborator contributing to extraction and sequence 67 of the isolates at University of Liverpool.

❖ Dr. Abdul Karim Sesay, Ms Jarra Manneh and Mr. Abdoulie Kanteh assisted in providing training and supervision on the library preparation and whole genome sequencing using the Illumina Miseq platform

❖ Dr. Jody Phelan provided training and support on the bioinformatic and phylogenetic analysis.

❖ Mr. Abdul Khalie Muhammad provided training on the statistical analysis.
**TABLE OF CONTENTS**

Abstract ........................................................................................................................... ii
Acknowledgement ........................................................................................................... iii
Dedication ......................................................................................................................... iv
Statement of work performed by Supervisors and colleagues ............................................. v
List of Tables ................................................................................................................... ix
List of Figures ................................................................................................................ x
Abbreviations ................................................................................................................ xi
Publications listed in appendix ....................................................................................... xii
  Original publication from this thesis: ................................................................. xii
  Other relevant publications giving background to the study ........................................ xii
Scientific conference attendance and presentations during the period: ............................ xiii

Chapter One: Introduction and review of literature ........................................................... 15
  Background: Overview of NTS .................................................................................. 15
  Nomenclature/Taxonomy: ............................................................................................ 17
  Laboratory identification .............................................................................................. 22
  Clinical presentation .................................................................................................... 22
  Burden and case fatality ............................................................................................... 23
  Transmission and risk factors ...................................................................................... 24
  Pathogenesis and virulence .......................................................................................... 25
  Antimicrobial resistance ............................................................................................... 27
  Vaccine development .................................................................................................... 28
  Comparative genomics of *Salmonella enterica* .......................................................... 29
  Genotypic adaptation of iNTS ...................................................................................... 30
  Control, prevention and treatment .............................................................................. 31
  Epidemiology and NTS disease burden in The Gambia ................................................ 32
Incidence and risk factors for NTS disease in The Gambia .......................................................... 33
Transmission of NTS in The Gambia.............................................................................................. 33
Serovar prevalence and Sequence types....................................................................................... 34
Antimicrobial resistance in Gambian NTS isolates....................................................................... 35
Problem statement ......................................................................................................................... 35
Justification: .................................................................................................................................... 36
Hypothesis: ....................................................................................................................................... 37
Overall aims ........................................................................................................................................ 37
Specific objectives: .......................................................................................................................... 37
Chapter 2. Materials and methods.................................................................................................. 38
Study setting and population .......................................................................................................... 38
Ethical approval: ............................................................................................................................. 39
Bacterial isolates: ............................................................................................................................. 39
Microbiological procedures............................................................................................................ 39
Antimicrobial susceptibility testing............................................................................................... 40
Genomic DNA extraction................................................................................................................ 40
Whole Genome Sequencing (WGS)............................................................................................... 41
Genome assembly and in Silico analysis ....................................................................................... 41
Phylogenetic analysis ..................................................................................................................... 42
Data management and statistical analysis .................................................................................... 43
Chapter 3: Results ........................................................................................................................... 44
Isolate source, associated disease syndrome and regional serovars differences....................... 44
Sequence types and eBurst groups ............................................................................................... 46
AMR genes, AMR phenotypes, plasmid replicons and virulence genes ...................................... 47
Phylogenetic analysis ..................................................................................................................... 52
Chapter 4: Discussion ..................................................................................................................... 57
LIST OF TABLES

Table 1. Cut-off values applied for interpreting zones sizes (mm) of the antimicrobials .......................... 40
Table 2. Baseline characteristics of Gambian non-typhoidal Salmonella disease patients from whom isolates were cultured for use in this study .................................................................................................................. 44
Table 3. Patients with multisite NTS simultaneous infections ................................................................... 44
Table 4. Gambian non-typhoidal Salmonella serovar distribution by disease among the isolates in this study .................................................................................................................................................. 45
Table 5. Gambian non-typhoidal Salmonella serovar distribution and disease prevalence ..................... 46
Table 6. eBurst groups of the major Gambian NTS serovars responsible for clinical disease ............... 47
Table 7. Summary of genotypic and phenotypic characteristics of serovars with resistance genes ....... 48
Table 8. Odds ratio of S. Enteritidis MDR against all NTS serovars .......................................................... 50
Table 9. Summary of plasmid replicons and serovar harbouring them in Gambian non-typhoidal Salmonella isolates ......................................................................................................................................... 51
Supplementary table other serovars ........................................................................................................ 84
LIST OF FIGURES

Figure 1. *Salmonella* colonial and microscopic morphology. a. *Salmonella* producing H₂S on deoxycholate citrate agar (DCA), b. Gram stain reaction as seen by optic microscopy and c. *Salmonella* viewed under electron microscopy (coloured) ................................................................. 18

Figure 3. Workflow showing analysis pipeline for genome sequence reads analysis pipeline. ........ 21

Figure 5. Map of The Gambia (inset map of Africa showing the geographic position of The Gambia within red box) showing the western (blue box) and eastern (burgundy box) regions...................... 38

Figure 6: Summary of the DNA extraction and sequencing workflow ......................................... 43

Figure 8: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of the samples...... 49

Figure 9a: Maximum likelihood genome phylogeny of *Salmonella Enteritidis* (NCBI: txid149539) from across Africa......................................................................................................................... 53

Figure 9b: Phylogenetic tree showing presence and absence of antimicrobial resistance-associated genes of *S. Enteritidis* within the West African clade.............................................................................. 54

Figure 10: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of *S. Typhimurium* isolates showing plasmids present within each isolate using the *S. Enteritidis* P125109 reference genome showing disease type, source region within Gambia, for each isolate. The tree was built using maximum-likelihood methods implemented in IQ-TREE followed by mid-point rooting ...................... 55

Figure 11: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of *S. Typhimurium* isolates showing plasmids present within each isolate using the *S. Typhimurium* LT2 reference genome showing disease type, source region within Gambia, for each isolate. The tree was built using maximum-likelihood methods implemented in IQ-TREE followed by mid-point rooting......................... 56
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>AMR</td>
<td>Antimicrobial resistance</td>
</tr>
<tr>
<td>ART</td>
<td>Antimicrobial resistant testing</td>
</tr>
<tr>
<td>AST</td>
<td>Antimicrobial susceptibility testing</td>
</tr>
<tr>
<td>API</td>
<td>Analytical profile index</td>
</tr>
<tr>
<td><strong>catA1/A2</strong></td>
<td>chloramphenicol acetyl transferase A1/A2</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory Standard Institute</td>
</tr>
<tr>
<td>DCA</td>
<td>deoxycholate citrate agar</td>
</tr>
<tr>
<td>GEMS</td>
<td>Global Enteric Multicentre Study</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency virus</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-drug resistant/resistance</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller Hinton Agar</td>
</tr>
<tr>
<td>MLST</td>
<td>MultiLocus Sequence Typing</td>
</tr>
<tr>
<td>NTS</td>
<td>Non typhoidal <em>Salmonella</em></td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulse-Field Gel Electrophoresis</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td><em>Salmonella enterica</em> subspecies <em>enterica</em> serovar Enteritidis</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td><em>Salmonella enterica</em> subspecies <em>enterica</em> serovar Typhimurium</td>
</tr>
<tr>
<td>SISTR</td>
<td><em>Salmonella In Silico</em> Typing Resource</td>
</tr>
<tr>
<td>spv</td>
<td><em>Salmonella</em> virulence plasmid</td>
</tr>
<tr>
<td>sSA</td>
<td>sub-Saharan Africa</td>
</tr>
<tr>
<td>TSI</td>
<td>Triple sugar iron</td>
</tr>
<tr>
<td>XLD</td>
<td>xylose-lysine-deoxycholate</td>
</tr>
</tbody>
</table>
**PUBLICATIONS LISTED IN APPENDIX**

**ORIGINAL PUBLICATION FROM THIS THESIS:**

**OTHER RELEVANT PUBLICATIONS GIVING BACKGROUND TO THE STUDY**
SCIENTIFIC CONFERENCE ATTENDANCE AND PRESENTATIONS DURING THE PERIOD:


2. Expanding International Research Collaboration on Antimicrobial Resistance: Towards a European One Health AMR Partnership. Virtual conference by JPIAMR-3rd Feb 2022 13:00-16:30

3. Antimicrobial Chemotherapy Virtual conference by GARDP and BSAC- 2nd and 3rd Feb 2022, 10:30 -14:10

4. How can mathematical and statistical models combine with big data to improve our response to pandemics? Virtual conference/symposium by LSHTM, 15:30-19:00 2nd Feb 2022

5. AMR INSIGHTS virtual workshop 19th Jan 2022


8. Improving Global Health by Strengthening Laboratory Capacity in Africa. 2nd Regional Conference for the West African Postgraduate College of Medical Laboratory Science (WAPCMLS). November 2021 virtual


10. Annual General and scientific meetings of the 44th & 45th West African College of Physicians (WACP). Themes: Universal Health Coverage, The role of vaccines in Universal health coverage and De-stigmatizing Mental Health in the sub-region 1-3 November, virtual

12. Laboratory Management 3-part Series, **WHO Public Health Laboratories knowledge sharing webinars. Sep/Oct 2021**

13. Drug resistant infections: causes, consequences, and considerations. **Royal Society Tropical Medicine and Hygiene Annual meeting** 11-12 October 2021, virtual. Attendance only


16. **Wellcome Trust Applied Bioinformatics & Public Health Microbiology** conference May 2021; online. Attendance only


CHAPTER ONE: INTRODUCTION AND REVIEW OF LITERATURE

BACKGROUND: OVERVIEW OF NTS

Non-typhoidal *Salmonellae* (NTS) are a common cause of foodborne gastroenteritis and severe disseminated infections dependent on the pathogen virulence and the host immune status (Feasey & Hadfield, 2016; Jones & Ingram, 2008). NTS are highly diverse consisting of over 2800 serovars and have a broad host range with most human infections (99%) caused by a small proportion of serovars (Gal-Mor & Boyle, 2014). Whilst many serovars are non-host restricted referred to as generalists such as *S*. Enteritidis and *S*. Typhimurium, a few are host adapted such as *Salmonella enterica* subspecies *enterica* serovar Dublin (*S*. Dublin) in cattle. Furthermore, a few others are host restricted such as *Salmonella enterica* subspecies *enterica* serovar Gallinarum (*S*. Gallinarum) in poultry and *Salmonella enterica* subspecies *enterica* serovar Abortusequi (*S*. Abortusequi) in horses.

The clinical characteristics of non-typhoidal salmonellosis endemic in SSA are that of a changing disease pattern, from gastroenteritis to invasive disease with a case fatality rate of between 20% to 50% (Ao & Feasey, 2015; Feasey & Hadfield, 2016; Okoro & Kingsley, 2012). A recent study found that invasive infections caused by NTS were responsible for around 49,600 deaths in SSA every year and many of these deaths were caused by multi-drug resistant (MDR) variants of the serovars *S*. Typhimurium and *S*. Enteritidis which have adapted to occupy the immunocompromised human niche (Stanaway & Parisi, 2019). These variants responsible for invasive disease are specialised lineages of *S*. Enteritidis and *S*. Typhimurium, respectively, and are distinct from those from other parts of the world (Crump & Heyderman, 2015; Feasey & Hadfield, 2016; Kingsley & Msefula, 2009). These lineages do not illicit gut inflammation and show a clear difference in disease manifestation, mainly an invasive lifestyle, and adapted to the immunosuppressed human niche (Feasey & Hadfield, 2016; Okoro & Kingsley, 2014). In addition, recent evidence emerging has shown that these variants also cause gastroenteritis and are carried asymptotically globally (Almeida & Seribelli, 2017; Ashton & Owen, 2017; Kasumba & Pulford, 2021) contrary to what was previously shown that they were restricted to invasive diseases in SSA (Okoro & Kingsley, 2014). However, molecular characterisation has differentiated isolates causing bacteraemia in SSA from those causing gastroenteritis in other parts of the world as being genetically distinct (Ashton & Owen, 2017; Pulford & Perez-sepulveda, 2021). Whole genome sequencing (WGS) has provided insight into the host adapted signatures associated with pathogenicity and metabolism especially for *S*. Typhimurium lineages and *S*. Enteritidis clades.
characterised by genomic degradation, a novel prophage and antimicrobial resistance repertoire (Feasey & Hadfield, 2016; Okoro & Barquist, 2015).

The lineage of S. Typhimurium causing MDR invasive disease confined mainly within HIV patients in sSA is S. Typhimurium ST313 (Okoro & Kingsley, 2012, 2014). The closely related S. Typhimurium ST313 lineages I and II evolved independently around 52 and 35 years ago with the acquisition of the cat chloramphenicol resistance gene (Okoro & Kingsley, 2012). Both lineages have been shown to carry the pSLT virulence plasmid, which also encodes for MDR genes conferring resistance to commonly used antimicrobials such as tetracycline, sulfamethoxazole-trimethoprim and chloramphenicol (Okoro & Barquist, 2015; Okoro & Kingsley, 2012). Although S. Typhimurium ST313 was considered to be confined to invasive disease in sSA, recent data has shown pan-susceptible variants of ST313 to be isolated from food and gastroenteritis globally (Almeida & Seribelli, 2017; Ashton & Owen, 2017; Kasumba & Pulford, 2021). In addition, a recent study in the Democratic Republic of Congo provided further evidence of evolution within ST313 clade causing invasive disease, where sub lineage of ST313 lineage II.1 harbouring IncHI2 plasmid and exhibiting extensive drug resistance was identified (Van Puyvelde & Pickard, 2019). Another study reported an additional pan susceptible lineage III (ST313 L3), an intermediate between lineages I and II that emerged in 2016 from Malawi (Pulford & Perez-sepulveda, 2021) providing further evidence of NTS evolution. Further molecular analysis of the NTS from the GEMs (Global Enteric Multicentre Study) population-based case-control study confirmed the presence of ST313 asymptomatic carriage and in diarrhoeal patients further highlighting that the sequence type is not only associated with invasive disease and suggesting anthropoontic transmission (Kasumba & Pulford, 2021). This MDR included decreased ciprofloxacin susceptibility, resistance to azithromycin, and extended spectrum beta-lactams.

S. Enteritidis also displays niche plasticity causing egg associated gastroenteritis and invasive disease caused by two related but phylogenetically different epidemic MDR clades of S. Enteritidis, West African and Central/Eastern African clades, respectively. These MDR clades are characterized by genome degradation carrying chloramphenicol resistance genes catA1 and catA2 respectively, plus a virulence tra gene (Feasey & Hadfield, 2016). These two clades are restricted in sSA and emerged around 1933 and 1945, respectively.

The use of antimicrobials such as fluoroquinolone, azithromycin and cephalosporins as drugs of choice for treatment is thus threatened by emerging MDR (Kalonji & Post, 2015; Lunguya & Lejon, 2013).
Compounding the problem is that these MDR genes are usually carried on conjugative plasmids, increasing risk of spread of resistance thus posing treatment challenge (Kariuki & Onsare, 2015; Mahon & Fields, 2016). An additional problem in managing NTS in sSA is the lack of effective diagnostics for timely and effective treatment. In the context of antimicrobial resistance (AMR), high morbidity and mortality and poor availability of diagnostics, a vaccine against iNTS is urgently needed as a long-lasting preventive strategy and hence continued research into the area is of great importance. Vaccines targeting both iNTS and typhoid are particularly important for sSA with a high burden of both (Kariuki & Mbae, 2019; Tennant & MacLennan, 2016)

**NOMENCLATURE/TAXONOMY:**

*Salmonella* genus was named after Daniel E. Salmon who first isolated the bacteria from porcine intestines in 1885 together with his assistant, Theobald Smith (Tindall, Grimont, & et al., 2005). It is a flagellated (Figure 1) Gram-negative, glucose fermenting, non-spore forming motile rod belonging to Enterobacterales. The taxonomic classification and nomenclature based on phenotypic traits has been controversial for many years until DNA-DNA hybridisation techniques resolved the dispute. Hence, the Judicial Commission of the International Committee for Systematics of Prokaryotes in 2005 adopted for the genus *Salmonella* to be assigned two distinct species, *S. bongori* (V) and *S. enterica*. The latter being further divided into six well known subspecies; I (*enterica*), II (*salamae*), IIIa (*arizonae*), IIIb (*diarizonae*), IV (*houtenae*) and VI (*indica*) (Figure 2) (Achtman & Wain, 2012; McQuiston & Herrera-Leon, 2008). Furthermore, an additional subspecies, VII, previously assigned to subspecies IV was determined on the basis of multilocus enzyme electrophoresis (Boyd & Wang, 1996). Further classification of subspecies into serovars is done using the Kauffman-White scheme of classification which is based on the antigenic determinants of the heat-stable somatic outer lipopolysaccharide (LPS) ‘O’ surface antigen encoded by the *rfb* gene and the flagellar ‘H’ antigen. The ‘H’ antigen is involved in the activation of host immune response encoded by the *fliC* and *fljB* genes for H1 and H2 respectively with the capacity to express either of the two proteins at a particular time in diphasic forms or only one protein in monophasic forms (Ryan & O’Dwyer, 2017). Hence antigenic formulae are represented by three positions for the O, H1 and H2 in the format O:H1:H2 differentiates *Salmonella* into more than 2800 serovars (Grimont & Weill, 2007).
Serovar designations based on antigenic formulae are useful for epidemiological investigation and ease of language. However, recent molecular studies have confirmed that they are not always necessarily genetically related due to limitation on use of epitopes (Achtman & Wain, 2012). Conversely, a few serovars lack expression of surface antigens thus limiting the use of serological serotyping. In addition, horizontal gene transfer is common contributing to genetic variation, thus the need for methods that recognise genetically related populations. Moreover, the low throughput, high expense, vast experience, and large collections of antisera required for traditional serotyping have necessitated quicker and more cost-effective alternatives. Thus, the emergence of molecular methods such as Pulse-Field Gel Electrophoresis (PFGE) and MultiLocus Variable number of tandem repeats Analysis (MLVA) to increase throughput and discriminatory power of Salmonella differentiation got recommended as alternatives to serotyping. These methods are especially useful for identifying outbreaks but insufficient to reliably assign serovars and difficult to standardise (Achtman & Wain, 2012). Other typing methods, such sequencing of the 16S rRNA gene or MultiLocus Sequence Typing (MLST) based on the sequence of seven-housekeeping genes distinguish clonal relationship but are ineffective in reliably differentiating closely related isolates (Achtman & Wain, 2012; Grimont & Weill, 2007). Thus, the use of the pan-genome in discriminatory and clustering of isolates using WGS for related isolates termed clonal complexes (eBurst group) allow linking several sequence types based on pattern of descent (Achtman & Wain, 2012; Alikhan & Zhou, 2018). With increasing accessibility, ease of use and reducing cost, the use of WGS is increasingly becoming popular and effective (Banerji & Simon, 2020;
Ibrahim & Morin, 2018) making it a quick replacement for traditional serotyping methods in routine diagnostic microbiology labs.
99% of human and animal infections caused by subspecie S. enterica.

**Genus Salmonella**

- **S. enterica**
  - **enterica** I
  - **salamae** II
  - **arizonae** IIIa
  - **diarizonae** IIIb
  - **houtenae** IV
  - **indica** VI

- **S. bongori** VII

Typhoidal serovars are human host restricted while non-typhoidal serovars are much diverse and infect both humans and animals. Differentiation into serovars is based on combinations of O, H and Vi antigens.

Disease syndrome

Typhoid fever

Gastroenteritis extraintestinal (bacteraemia & other focal infection)

**Figure 2:** General overview of the current classification of Salmonella enterica adapted from Achtman & Wain, 2012; McQuiston & Herrera-Leon, 2008)
Genome-based serotyping is now regarded as the gold standard for serotyping and can be used to enhance epidemiological surveillance and identify phylogenetic relationships of closely related species and isolates (Armstrong & MacCannell, 2019; Banerji & Simon, 2020; Pornsukarom & Van Vliet, 2018). The method, however, depends on the use of high-quality genomic data to determine the conserved or core-genome and variable genes for investigating evolution and population structure. Online bioinformatic typing tools in curated species-specific platforms such as Salmonella In Silico Typing Resource (SISTR) and SeqSero for in silico prediction of assembled and raw sequence reads respectively has made analysis more accessible for non-bioinformaticians (Banerji & Simon, 2020; Ibrahim & Morin, 2018; Yoshida & Kruczkiewicz, 2016). These tools use genoserotyping, MLST, rMLST and cgMLST increasing sensitivity to >99% making WGS data analysis widely applicable (Banerji & Simon, 2020; Ibrahim & Morin, 2018). Furthermore, the addition of genome data to national and international Salmonella public health and food protection databases such as PulseNet and Genome Tracker has allowed tracking of disease outbreaks more efficiently (Armstrong & MacCannell, 2019).

Genome analysis has shown that more than 10% of Salmonella serovars are either polyphyletic (serovars with genetically distinct lineages e.g.; S. Virchow, S Bredeney, S Livingstone, S Stanleyville and S. Newport) making conventional methods limited in their ability to distinguish them; or paraphyletic (distinct serovars that are genetically closely related, for example; S. Enteritidis, S. Dublin, S. Nitra, S. Gallinarum and S. Berta (Achtman & Wain, 2012; Zhang & Payne, 2019). In addition, genomic surveillance has identified characteristics of host adapted lineages causing invasive disease in sSA emerging with new pathogenesis (Feasey & Dougan, 2012; Feasey & Hadfield, 2016). However, a critical challenge with genomic data is the computing requirement and large databases to store and analyse data for which cloud technologies are being developed. The workflow of analysis is shown in in Figure 3.

Figure 3. Workflow showing analysis pipeline for genome sequence reads.
LABORATORY IDENTIFICATION

Techniques include using metabolic, biochemical and serological techniques for identification up to serovar level. In many routine labs, colonies are grown from solid media such as MacConkey, XLD, *Salmonella-Shigella* agar, and Hektoen enteric agar from various samples such as stool, blood or urine and incubated overnight. Morphological inspection for hydrogen sulphide production shown by black centres on the colonies, lack of lysine decarboxylase production on XLD agar gas production, dextrose fermentation and glucose fermentation on slopes such as triple sugar iron (TSI), and non-fermentation of lactose on MacConkey agar further corroborate the identification of the pathogen. Alternatively, the use of API 20E diagnostic combines 20 biochemical substrate reactions to identify *Salmonella* from suspected colonies to species level. In addition, recent advances in microbiological identification techniques such as the use of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry only identifies *Salmonella* to species level (Bizzini & Durussel, 2010). Although the initial cost of the instrument is high, the cheaper running cost makes it a great alternative to the use of biochemical methods (Rychert, 2019). The phenotypic confirmation to serovar level however, remains only through the use of specific antisera (Fábrega & Vila, 2013).

CLINICAL PRESENTATION

In humans, NTS most often presents as self-limiting gastroenteritis with an incubation period between 6-12 hours lasting up to 10 days rarely requiring antimicrobial treatment (Eng & Pusparajah, 2015; Gal-Mor & Boyle, 2014). However, infections in the immunocompromised patients and invasive infections such as bacteremia and meningitis require urgent antimicrobial treatment with azithromycin, ciprofloxacin or the 3rd generation cephalosporins (Crump & Sjölund-Karlsson, 2015). In addition, NTS cause other focal infections. Asymptomatic carriage and shedding of *Salmonella* following the resolution of clinical disease is also common in children under five years and those treated with antimicrobials (Gal-Mor & Boyle, 2014), providing reservoirs for potential transmission and possible re-infection in the community (Acheson & Hohmann, 2001).

Invasive NTS however, has a markedly different presentation, closer to enteric fever in its clinical presentation than standard NTS disease (Gal-Mor & Boyle, 2014). Patients present with febrile illness, accompanied by cramping and vomiting with or without diarrhoea, a similar presentation of other enteric infections and malaria making differential diagnosis a challenge (Fábrega & Vila, 2013; Feasey & Dougan,
2012). Regional serovar differences are observed but *S. Typhimurium* and *S. Enteritidis* are consistently implicated as the main cause of disease in human infections (Eng & Pusparajah, 2015). This observation is borne out in the African context, with these two serovars being predominant in iNTS, albeit with marked pathotypes (Kwambana-Adams & Darboe, 2015; Morpeth & Ramadhani, 2009).

**Burden and Case Fatality**

NTS remain a major global public health threat causing salmonellosis despite extensive efforts to reduce burden which is due partly to their adaptability, host susceptibility, virulence and other environmental factors (Cheng & Eade, 2019). The global annual burden of enteric NTS was estimated at 94 million cases and 155,00 deaths with an all incidence of 1,140 per 100,000 (Majowicz & Musto, 2010). Africa accounts for circa 2.4 million cases, with an incidence of 320/100,000 and 4,100 deaths (Majowicz & Musto, 2010); a comparably lower burden when compared to other parts of the world (Jajere, 2019; Majowicz & Musto, 2010). In sharp contrast, the global burden of iNTS in 2010 was estimated at 3.4 million cases, with a case fatality rate of 20%, all incidence of 49 per 100,000 and mortality of 681,316 (Ao & Feasey, 2015). Africa accounted for 55% of cases with an incidence of 227 per 100000. In addition, age specific incidence differences were noted for children <5years and 30-35 years mostly affected. Despite the relatively lower prevalence of NTS gastroenteritis in Africa compared to the rest of world, the additional burden caused by invasive NTS (iNTS) disease on this continent makes it the continent with the highest NTS burden (Gal-Mor & Boyle, 2014). Notwithstanding, a more recent more comprehensive study by the Global Burden of Disease (GBD) in 2017 estimated 535,000 illnesses and all invasive NTS incidence of 34.5 per 100,000 resulting in 77,500 deaths. (Stanaway & Parisi, 2019). This is significantly lower than the previously reported estimated burden of over half a million deaths. This study also reported an overall case fatality rate of 14.5% lower than 20-25% previously reported. In addition, this study estimated gastroenteritis burden at 95.1 million illnesses resulting in 50,800 deaths, higher than previous mortality attributable to gastroenteritis. Despite these conflicting estimates, iNTS remains a major cause of death in those under 5 years, HIV infected adults and malaria in sSA having implications on management of patients as diagnostics capabilities remain scarce in many settings.
TRANSMISSION AND RISK FACTORS

Animals are the major reservoir of NTS, with warm blooded animals being the predominant reservoir of disease-causing subspecies I (Tindall et al., 2005). In Africa, although NTS has been isolated from agricultural animals like cattle, goats, sheep, pigs, camels and poultry (Morpeth & Ramadhani, 2009; Thomas, de Glanville, & et al., 2020; Wilson & Pulford, 2020), a link between zoonotic transmission and disease is yet to be established (Dione & Ikumapayi, 2011; Kariuki & Revathi, 2006; Kotloff & Nataro, 2013). A recent study from sSA provides evidence for person-to-person transmission as a source of transmission for invasive NTS (Post & Diallo, 2019). However, food contamination is considered the major source of infection globally due to NTS ability to survive in food such as meat, eggs, dairy products and vegetables (Afema & Byarugaba, 2016; de Freitas Neto & Penha Filho, 2010). Such foodborne outbreaks of NTS gastroenteritis have been reported from sSA (Morpeth & Ramadhani, 2009) and a recent genomic study in Ghana described a possible transmission through the food chain (Aldrich & Hartman, 2019). Furthermore, a study in Uganda confirmed that Salmonella transmission in plants is also driven by its ability to survive and replicate in plants using different mechanisms (de Moraes & Desai, 2017), which may enable transmission (Silva & Puente, 2017). Infections in Africa may be seasonal with peaks of infection during the wet season (Morpeth & Ramadhani, 2009). While the reasons for seasonality are not conclusively determined, increased waterborne infections are a likely risk factor. The risk factors for NTS gastroenteritis in Africa are not well characterised and has increased the difficulty in instituting evidence based public health interventions to reduce disease incidence (Morpeth & Ramadhani, 2009).

Multiple host risk factors, however, are described for iNTS; these include extremes in age (young age 6-36 months and elderly), co-infections with HIV/AIDS in adults and malaria infection, co-morbidities with malnutrition and sickle cell disease (SCD) in children (Feasey & Dougan, 2012; MacLennan & Gondwe, 2008; Oneko & Kariuki, 2015). These morbidities are all endemic in sSA and hence reason for disease burden. The transmission of iNTS in developing countries is poorly understood, but considered to be through person-to-person transmission due to human host adaptation (Crump & Heyderman, 2015; Post & Diallo, 2019). However, host adaptation has been described with numerous studies having established that genomic degradation of iNTS, resulting in reduced gut inflammation in the infected host, is the main determinant for invasiveness associated with MDR (Feasey & Hadfield, 2016; Okoro & Kingsley, 2014). The reason for different adaptations and disease presentation is yet to be fully understood. Further to this, recurrent infection in the form of recrudescence and reinfection are a frequent occurrence and are associated with a marked increase in mortality, among individuals with untreated HIV (Ruby & McLaughlin, 2012).
recent study reported that clinical isolates of both *S. Enteritidis* and *S. Typhimurium* induced persister status in human macrophages following treatment with antimicrobials by inhibiting translation of acetylation of aminoacyl-tRNAs, thus, leading to clonal relapse following treatment in up to half of infected patients (Rycroft & Gollan, 2018).

**PATHOGENESIS AND VIRULENCE**

Ingested *Salmonella* activates its acid tolerance response as a defence mechanism whilst passing through the gastric acid of the stomach before reaching the intestinal lumen and attaching to the mucosa for invasion through the cells of the epithelial lining (Figure 4) (Fàbrega & Vila, 2013). Penetration occurs through M cells and enterocytes or via direct uptake by dendritic cells from the sub-Mucosa. The pathogenic success of NTS serovars is directly linked to its plethora of virulence factors to control its ability to infiltrate, adhere and colonize the host cells. These virulence factors include flagella, fimbriae, capsule, toxins, pathogenicity islands in the chromosome and associated plasmids (Cheng & Eade, 2019). In addition, severity of disease is aided by host susceptibility, serovar fitness, infectious dose, and antimicrobial resistance. *Salmonella* pathogenicity islands (SPIs) are gene clusters encoding several effector proteins such as those of the *Salmonella* type III secretion system (T3SS) encoded by SPI-1 and virulence factors located on other parts of the chromosome (Marcus & Brumell, 2000). They are widespread and 24 have been identified to date, with SPI-1 to -5 being the most prevalent considered acquired through horizontal gene transfer and responsible for virulence determinants. Whilst most SPIs are found in most serovars, a few are serovar restricted (Ramos-Morales, 2012). The T3SS includes multi-channel proteins which combine to produce an effector apparatus that inject effector proteins such as SipA, SipC, SopB/SigD and others into the cytoplasm of the host cell. In addition, SPI-1 harbours the genes responsible for invasion whilst SPI-2 harbours the genes for intracellular replication and survival (Cheng & Eade, 2019; Marcus & Brumell, 2000).
With the exception of *S. Gallinarum* and *S. Pullorum*, the flagella allow motility and chemotaxis towards host epithelial layers thus important inducers of host immune response (Fàbrega & Vila, 2013). Within host cells, *Salmonella* become enclosed in *Salmonella*-containing vacuoles (SCV) which they survive and replicate thus evading the host. Uptake by resident macrophages leads to dissemination into the bloodstream accumulating in mesenteric lymph nodes. Fimbriae also aid in attachment and adhesion with variations seen in different serovars. *Salmonella* plasmids associated with virulence known collectively as pSV enhances pathogenicity and also found mainly inserted in the chromosome of subspecies II, IIIa, IV and VII (Che‌ng & Eade, 2019). They are found in only a few clinically important serovars of subspecies I such as *S. Typhimurium* (pSLT/pSTV), *S. Enteritidis* (pSEV), *S. Dublin* (pSDV), *S. Gallinarum* (pSPV), *S. Choleraesuis* (pSCV); aiding in intra-macrophage survival, invasion and adaptation to host (Silva & Puente, 2017). The sizes of the plasmids range from 50kb in *S. Choleraesuis* to 285 kb in *S. Sendai*, with the signature locus conferring the virulent phenotype found in the 7.8 kb *spv* (*Salmonella* virulence plasmid) region/operon (Cheng & Eade, 2019; Marcus & Brumell, 2000; Silva & Puente, 2017). Although its sole role in pathogenicity remains unclear and controversial as serovars lacking pSV have been found to be equally virulent, loss of the *spv* region in those harbouring them results in loss of the virulence phenotype (Silva & Puente, 2017). However, in ST313, the presence of pSV serovar-specific virulence plasmids

---

**Figure 4.** Pathogenesis of *Salmonella enterica* serovar Typhimurium from (Fàbrega & Vila, 2013). 1; *Salmonella* attaches to the intestinal lumen using adhesins, encoded within pathogenicity islands. 2; and 3; Invasion and engulfment by M cells or directed through the lumen is mediated by virulence factors. 4; Alternatively, bacterial cells can also be directly taken up by dendritic cells from the submucosa. 5; inside the cytoplasm, *Salmonella* is localized within the *Salmonella* containing vacuole SCV, where it replicates. 6; The SCVs then release the internal cells to the submucosa. 7; Bacteria are internalized within phagocytes. These infected phagocytes can disseminate through the lymph and the bloodstream adapted from: (Fàbrega & Vila, 2013)
harboured by S. Typhimurium known as pSLT-BT, has a role in invasive pathogenesis which functions by inhibiting inflammation and encoding genes which affect MDR (Schroeder & Brooks, 2017).

**ANTIMICROBIAL RESISTANCE**

The emergence and spread of antimicrobial resistance in *Salmonella* remains a global public health concern both in human and animal health leading to poorer clinical outcomes. The misuse of antimicrobials induces selective pressure and heighten resistance thus posing a risk of spread of MDR (Pornsukarom & Van Vliet, 2018; van Boeckel & Pires, 2019). The additional threat for zoonotic transmission is posed by the use of antimicrobials in feed as growth promoters and prophylaxis in animals (Eng & Pusparajah, 2015). Varied Surveillance data has shown increasing MDR to first line antimicrobials such as ampicillin, chloramphenicol and sulfamethoxazole/trimethoprim are common (Kariuki & Gordon, 2015; Tadesse, Ashley, & et al., 2017) leading to less use of these drugs. In addition, emerging resistance in cephalosporins and decrease susceptibility to fluoroquinolones as alternatives is particularly worrisome raising the threat of highly resistant isolates which warrants need for alternatives for treatment of invasive disease (Van Hoek & Mevius, 2011). Resistance is usually carried on conjugative plasmids (some of which are serovar-specific) placed within transposons and are thus posing a threat to treatment and dissemination of MDR phenotypes (Van Hoek & Mevius, 2011). In addition, integrons or Integrative and conjugative elements (ICEs) can also mediate resistance and hence strategies to combat resistance may include but not limited to phage therapy, combination therapy, changing delivery route and designing new ones (Sultan & Rahman, 2018). Furthermore, spreads of isolates containing hybrid plasmids poses an added challenge. Optimal treatment is successfully completed within 10 to 14 days. In the case of lack of susceptibility data, both cephalosporins and fluoroquinolone empiric treatment is warranted. This highlights the need for accurate diagnostics and the urgent need in sSA especially in the advent of AMR surveillance (Avdic & Carroll, 2014; Morency-Potvin, Schwartz, & Weinstein, 2017).

Mechanisms of resistance are employed by NTS ranging from enzyme production, activation of efflux pumps, reduced membrane permeability and modification of the cellular targets with implication for therapeutic failure. Therefore, choosing the right antimicrobial is essential to guide treatment and hence understanding resistance in *Salmonella* is necessary to detect emerging resistance. Culture-based phenotypic antimicrobial susceptibility testing (AST) methods has the ability to detect new and emerging detecting resistance but these methods are slow, breakpoints based on epidemiological data or mechanism
of resistance, and lack inter-laboratory standardization (Khan & Siddiqui, 2019). Hence, newer technologies that support rapid testing is essential in this ‘post antimicrobial era’ (Khan & Siddiqui, 2019). Genotypic testing has revolutionised diagnostics, surveillance and research with the ability to provide rapid and accurate prediction of all resistant genes from genomic data through antimicrobial resistance testing (ART) using multispecies databases such as ResFinder, MEGARes or CARD and species-specific ones such as SISTR or SeqSero for Salmonella (Su & Satola, 2019; Zankari & Hasman, 2013). Whilst ResFinder identifies acquired antimicrobial resistance and chromosomal mutated genes using BLAST, lacking the ability to differentiate functional integrity and resistance as a result of acquired variation in housekeeping genes (Zankari & Hasman, 2012). Other tools with differing scopes such and CARD is focused not only on acquired resistance but intrinsic and dedicated resistance genes (McArthur & Waglechner, 2013). The drawback of ART such as detection based on known resistance, need for expertise and sSA. In addition, the presence of some genes might not necessarily be phenotypically expressed due to mutation and in de novo assembly, a resistance gene may be missed if split occurs in multiple contigs (Su & Satola, 2019). A concordance of 95 % has been noted between the two that makes the use of WGS using online analysis tools such as ResFinder especially for surveillance a viable option (Zankari & Hasman, 2013).

VACCINE DEVELOPMENT

Developing NTS vaccines has proven difficult due to poor investment and political will partly as a result of a decline in malaria incidence and disease burden not fully appreciated (Balasubramanian & Im, 2019). Nonetheless, recent compelling evidence of a higher disease burden and case fatalities has prompted ongoing research. Evidence has been shown on the role for placental transferred maternal-specific IgG and potential IgA in breast milk in protecting infants during the life (De Alwis & Tu, 2019; MacLennan & Gondwe, 2008) suggesting for vaccines to be given between 2-4 months (De Alwis & Tu, 2019) correlating with waning immunity (Feasey & Dougan, 2012; MacLennan & Gondwe, 2008). Given children under years and the immune suppressed are mostly affected, vaccine candidates should target these groups. Developing an ideal vaccine has, however, proven difficult because NTS can survive both as intracellular and extracellular pathogens requiring both cellular and humoral immune responses (Gayet & Bioley, 2017; MacLennan & Martin, 2014; Tennant & MacLennan, 2016). In addition, NTS has diverse O and H antigens making vaccine development a challenge. Antibodies recognising surface polysaccharide correlate with protection in children, however these vaccine candidates are known to elicit weak immune response in children thus advocating for maternal immunisation as an alternative in this category for a delayed
immunisation at 9 months to one year (De Alwis & Tu, 2019). However, surface proteins are highly conserved and thus can be targeted for vaccine development. Several vaccine candidates are being developed focused on the few serovars responsible for the majority of disease such as S. Typhimurium, S. Enteritidis and S. Dublin (Balasubramanian & Im, 2019). In addition, bivalent vaccines targeting S. Typhimurium and S. Enteritidis are also in clinical trials as well as trivalent vaccines including S. Typhi. Promising evidence on the efficacy is emerging on mucosal vaccines composed of supernatant-derived SPI-2 (component vaccine for reducing colonisation) (Ferreira, 2015) and live attenuated subunit oral vaccines candidates conferring protection against most serovars by deletion of the phosphotransferase genes ptsI and crr (Zhi & Lin, 2019).

**Comparative Genomics of Salmonella enterica**

Increased availability of genomic sequences has offered insight into greater variation than previously predicted. *Salmonella* serovars were historically categorised as a single species *S. enterica*, until sequence analysis differentiated the second species *S. bongori*. Analysis of *Salmonella* genomes has shown that they are highly conserved displaying high amino acid similarity (~99% identity) and a core stable set of about 3400 gene families (Wassenaar & Jun, 2017). The seven MLST housekeeping genes for *Salmonella, aroC, dnaN, hemD, hisD, purE, sucA* and *thrA* ([https://pubmlst.org/bigsdb?db=pubmlst_mlst_seqdef&page=schemeInfo&scheme_id=2](https://pubmlst.org/bigsdb?db=pubmlst_mlst_seqdef&page=schemeInfo&scheme_id=2), are found in the conserved part of the genome with little variation. MLST has a wide range of applications including evolutionary studies but it is less useful for differentiating closely related isolates (Achtman & Wain, 2012). The use of antigenic formulae is not reliable in assigning serovars as genes encoding epitopes can be transferred through horizontal gene transfer and homologous recombination. This can result in genetically related serovars having different epitopes and unrelated serovars having similar alleles (Achtman & Wain, 2012). However, the addition of ribosomal MLST genes in identifying genetic-linked clusters known as e-Burst groups (eBGs) can be applied to increase resolution of isolates which are related by evolutionary descent (Achtman & Wain, 2012). Many eBGs correlate with serovars with exceptions of polyphyletic serovars such as *S. Weltevreden, S. Montivideo* and *S. Newport* have also been found to span multiple unrelated eBGs which may have public health implications in transmission and control.

The core genome (cgMLST) conserved in all members displays genomic variation and can reveal evolutionary rates and typing (Achtman & Wain, 2012; Alikhan & Zhou, 2018), albeit with low bootstrap
values resulting in uncertainty likely as a result of the large core genome. The region of the large highly conserved core genome has important functions vital for *Salmonella* and the smaller highly variable regions serve as good candidates for investigating genetic variation. However, sole use to construct trees is doubtful due to lack of consensus when they are used in isolation (Leekitcharoenphon & Lukjancenko, 2012) supporting the additional use of the pan-genome in construction trees. The pan genome does not only compare shared genes but also considers the absence of genes including accessory genes. Invasive NTS serovars that display pseudogenes and loss of genes like *S*. Typhi have decreased conserved genes in the core thus increasing the pan-genome. The pan-genomes have high bootstrap values provides excellent resolution and correlates with better with phenotype than phylogeny (Leekitcharoenphon & Lukjancenko, 2012). It has strong discriminatory and clustering capabilities hence excellent for epidemiological investigations although high quality sequence data from short reads is required.

**Genotypic adaptation of iNTS due to genome degradation**

The iNTS disease in sSA is caused mainly by serovars Typhimurium and Enteritidis both associated with MDR and novel prophages (Feasey & Dougan, 2012). Studies have shown genotypic differences between iNTS causing invasive disease and NTS limited to gastroenteritis in Africa. Gene acquisition events and selective regulatory evolution in the non-coding DNA region through horizontal acquisition contributes to pathogenicity (Ilyas, Tsai, & Coombes, 2017). In addition, within host evolution in the absence of horizontal gene transfer such as deletions and genomic rearrangements leading to genomic variation and host adaptation contribute to *Salmonella* pathogenesis (Tanner & Kingsley, 2018). Genomic degradation similar to those found in *S*. Typhi and acquisition of novel prophages have allowed for adaptation to a new niche, of the immunocompromised host during the ongoing HIV pandemic (Feasey & Hadfield, 2016; Okoro & Barquist, 2015). The mechanism of genomic degradation in the form of pseudogenes or gene deletion is a host-restricted signature evolving towards invasive disease (Okoro & Barquist, 2015) and increased pathogenicity is attributed to intracellular persistence and greater host vulnerability warranting extended treatment of iNTS for this category of patients (Gordon, 2008; Ruby & Mclaughlin, 2012).

The emergence and spread of the novel multi-drug resistant and highly virulent lineages of *S*. Typhimurium ST313 and *S*. Enteritidis clades across sSA has recently been reported (Feasey & Hadfield, 2016; Okoro & Kingsley, 2012). These closely related *S*. Typhimurium ST313 lineages I and II are considered to have evolved independently in close association with the emergence of the HIV pandemic. Both have been shown to carry the pSLT virulence plasmid, which also encodes for MDR to common antimicrobials such
as ampicillin, sulfamethoxazole-trimethoprim, and chloramphenicol. Both lineages I (~52) years and lineage II (~35 years) have been shown to carry the pSLT virulence plasmid, which also encodes for MDR and evolved from two different focal hubs driven by the HIV pandemic (Okoro & Barquist, 2015). Until recently, *S. Typhimurium* ST313 was considered to be restricted to invasive disease in sSA but evidence has shown that it was also associated with gastroenteritis elsewhere (Ashton & Owen, 2017). A recent population-based study in the rural eastern region found a low prevalence of ST313 pathovariant virulent lineage (Kanteh & Sesay, 2021), and the WGS data of from the GEMs study also found this lineage from asymptomatic carriers (Kasumba & Pulford, 2021). It is important to note that the clinical presentation of *S. Typhimurium* ST313 pathovariant is not restricted to MDR invasive disease as previously described as pan-susceptible lineages have also been recently isolated from cases of gastroenteritis and food surveys in other parts of the world (Almeida & Seribelli, 2017; Ashton & Owen, 2017). In addition, recent genomic analysis reported resistance to 3rd generation cephalosporins and fluoroquinolones in *S. Typhimurium* ST313, the recommended drugs of choice for invasive diseases. Genome evidence showed human-host restriction similar to *S. Typhi* with evidence of person-to-person transmission (Feasey & Dougan, 2012; Okoro & Barquist, 2015). The two epidemic clades of *S. Enteritidis*, the Central/East African and West African clade causing iNTS emerged around 1945 and 1933 and have similar niche plasticity (Feasey & Hadfield, 2016). These clades have acquired antimicrobial resistance genes with the West African clade carrying chloramphenicol acetyl transferase A1 (*cat*A1) and the Central/East African isolates carry *cat*A2 gene and tetracycline resistance *tet*(A).

**CONTROL, PREVENTION AND TREATMENT**

The global surveillance of NTS has provided crucial knowledge on emerging serovars, regional differences and patterns of transmission. For example, *S. Enteritidis* is known to be transmitted mainly through eggs and poultry products. In addition, data on serovar diversity and virulence determinants has shown differences in niche adaptation and within serovar variations. The understanding of serovar characteristics is important for targeted control requiring concerted efforts by all stakeholders in prevention of infection. Hence, efforts should be multifaceted including improvement in hygiene and sanitation which are effective in minimising transmission through animals and the food chain thus reducing the burden of NTS. Vaccination of populations at high-risk could also help reduce the burden of NTS disease. For those with self-limiting gastroenteritis, rehydration and resting are sufficient in NTS disease management with antimicrobial therapy being only indicated for the severely ill or high-risk patients (Fàbrega & Vila, 2013)
as well as in iNTS disease. In the HIV positive patient, the increased pathogenicity attributed to intracellular persistence and greater host vulnerability demands extended treatment of iNTS for this category of patients (Onoko & Kariuki, 2015). Since iNTS disease overlaps with malaria (Kariuki & Onsare, 2015; Muthumbi & Morpeth, 2015; Takem, Roca, & et al., 2014), patients at increased risk who test positive for malaria should be given antimicrobial prophylaxis for iNTS in endemic regions. Reduction in the incidence of malaria has proven to be an effective tool in the reduction iNTS disease. In addition, intestinal schistosomiasis has shown to increase invasive salmonellosis through attachment to the adult schistosomes and increases resistance to antimicrobials (Barnhill & Novozhilova, 2011; Hsiao & Toy, 2016). Hence, reduction in schistosomiasis could help control iNTS. The combined presence of iNTS, malaria, and schistosomiasis in endemic regions poses a challenge in the management of febrile patients with iNTS when present as diagnosis can be missed due to lack of blood cultures facilities unavailable in many parts of sSA. Therefore, combined management efforts are needed in the fight against iNTS. Surveillance, prevention, and treatment strategies remain paramount in local epidemiology and effective control.

Epidemiology and NTS Disease Burden in The Gambia

The burden of NTS disease in The Gambia remains high and NTS remains a leading cause of invasive disease across all age groups with an estimated prevalence of 0.8-1.0% in bacteraemia and a comparable lower prevalence for gastroenteritis at 0.1% (Darboe & Okomo, 2019; Enwere & Biney, 2006; Hill & Onyeama, 2007; Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015). By contrast, invasive salmonellosis was rarely caused by S. Typhi. A previous study over three decades ago found a high prevalence of both iNTS (71/259; 27.4%) mainly in young children and S. Typhi (45/259; 17.4%) in older patients respectively (Mabey & Brown, 1987). The reason for the drastic decline observed for typhoid prevalence is linked to improved hygiene and sanitation as humans remain the only known carriers of S. Typhi. A general decline in iNTS has also been reported to be associated with declining malaria incidence (G. Mackenzie & Ceesay, 2010). Notwithstanding, we recently reported it as a major pathogen causing bacteraemia in sickle cell patients and in all febrile patients (Darboe & Okomo, 2019; Soothill & Darboe, 2016). This is particularly significant as penicillin which is not a recommended drug for iNTS is given as a prophylaxis to patients with sickle cell disease. Thus, the re-evaluation of antimicrobial prophylaxis in this cohort with consideration given to the local resistance pattern is warranted. Importantly, cases of iNTS bacterial meningitis in paediatric patients less than 10 years old have been reported in The Gambia (Enwere & Biney, 2006) and elsewhere in sSA. This warrants NTS consideration in protocols for the appropriate
management of meningitis following post-vaccine declines in the prevalence of both *Haemophilus influenzae* type B and pneumococcal meningitis.

**INCIDENCE AND RISK FACTORS FOR NTS DISEASE IN THE GAMBIA**

The incidence of iNTS declined from 105 (1989-1991) to 29 (2008) per 100,000 population in the eastern region and from 60 (1979-1984) to 10 (2003-2005) per 100,000 population in the western region (G. Mackenzie & Ceesay, 2010). A population-based study conducted 15 years ago identified malaria, malnutrition and anaemia as important risk factors for the incidence of iNTS in The Gambia (Enwere & Biney, 2006). It is important to note, however that the fatality rate in The Gambia of 4.4% in children (Enwere & Biney, 2006) is lower than the 20-25% described in sSA (Eng & Pusparajah, 2015). The reason for this difference in clinical outcome needs further elucidation. Although not many studies on risk associated with NTS have been done in the Gambia, this population-based study also found that invasive NTS in children 2-29 month was strongly associated with diarrhoea (Enwere & Biney, 2006). Association with malaria has been collaborated as evidence of a decline of iNTS has been described with decreasing malarial incidence (G. Mackenzie & Ceesay, 2010). It is important to note however, that most studies on iNTS risk factors and incidence were done on children less than 5 years of age.

**TRANSMISSION OF NTS IN THE GAMBIA**

The reservoirs and transmission of NTS serovars in The Gambia is yet to be determined. Although few studies in sSA support evidence for zoonotic transmission (Afema & Byarugaba, 2016; Wilson & Pulford, 2020), a population-based case-control study done in the eastern region of The Gambia on NTS gastroenteritis a decade ago did not support zoonotic transmission among children and animals in close proximity (Dione & Ikumapayi, 2011). In addition, the Global Enteric Multicentre Study (GEMS) of acute moderate to severe diarrhoea among children under 5 years determined that NTS is prevalent, in both patients with diarrhoea and healthy controls highlighting transient carriage in our setting which is a potential source of transmission (Kasumba & Pulford, 2021; Kotloff & Nataro, 2013). Of particular interest is the seemingly site-specific serovar prevalence pattern and regional differences seen (Dione & Ikumapayi, 2011; Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015). Importantly, serovars seen in enteric cases were different from those causing invasive diseases. Although the difference in the serovars causing gastroenteritis as opposed to iNTS may be due to sampling bias, as most of the studies were done on patients presenting to hospital with obvious clinical syndromes, a previous population-based case-control study (Dione & Ikumapayi, 2011) in the same setting found similar serovars both in cases and the
control group. This further contradicts the hypothesis of faecal-oral route transmission and compounds the challenge in understanding the transmission of NTS in our setting. Thus, transmission exploring environmental factors and anthropogenic transmission may be crucial in the understanding of differences in transmission of NTS disease in The Gambia. The differences in the serovars in enteric and invasive sites is yet to be fully understood in our setting. It is therefore important that carriage studies on NTS are done, and further transmission dynamics investigated to provide insight into the transmission dynamics in our setting. Studies in sSA are yet to comprehensively provide evidence to support human to human transmission of iNTS (Afema & Byarugaba, 2016; Kariuki & Revathi, 2006) suggesting a possible link of food chain. However, a recent study (Post & Diallo, 2019) supports evidence for a human reservoir for the S. Typhimurium pathovariant lineage ST313. The advent of WGS and more robust epidemiological studies using WGS could establish transmission pathways (Kariuki & Revathi, 2006; Phoba & Barbé, 2020; Post & Diallo, 2019).

**SEROVAR PREVALENCE AND SEQUENCE TYPES**

This review has shown that *Salmonella* spp remain a major cause of disease with evidence of emerging MDR and higher prevalence of invasive NTS than S. Typhi. Regional differences in serovars show S. Typhimurium as the most prevalent in the western region as opposed to S. Enteritidis in the eastern region (Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015). A recent population-based study of invasive salmonellosis in the eastern region of the country has highlighted a decline in the proportion of S. Enteritidis which is being replaced by other serovars (Kanteh & Sesay, 2021). Notwithstanding, up to half of all infections with iNTS in The Gambia are caused by S. Enteritidis ST11 and S. Typhimurium ST19 (Darboe & Bradbury, 2020; Kanteh & Sesay, 2021) with the rest composed of other diverse serovars. In a study conducted five years ago, nearly half of serovars were non-typable due to our limited phenotypic serological typing. The purchase of antisera is guided by knowledge of circulated serovars and requires the use of 150 specific antisera and well-trained personnel to interpret results (Diep & Barretto, 2019) resulting in huge cost implications for most routine labs. The additional lack of detailed insight into the currently circulating serovars also makes purchasing required antisera a challenge. This highlights significance of the use of WGS in the characterisation of NTS (Banerji & Simon, 2020; Ibrahim & Morin, 2018; Zou & Li, 2016).

Although S. Typhimurium ST19 is known as a major cause of gastroenteritis globally (Majowicz & Musto, 2010), a pathovariant virulent lineage S. Typhimurium ST313 is the leading cause of multi-drug resistant
invasive disease confine mainly in sSA (Carden & Okoro, 2015; Okoro & Kingsley, 2014). Immunosuppression is an important predisposing factor for infection, with up to 50% mortality (Branchu & Bawn, 2018; Okoro & Kingsley, 2012). With changing disease pattern of NTS in sSA associated specific lineages of S. Typhimurium and S. Enteritidis, keeping up with constant changes remain a major concern and warrant surveillance (Feasey & Hadfield, 2016; Kingsley & Msefula, 2009; Mahon & Fields, 2016; Uche, MacLennan, & et al., 2017).

**ANTIMICROBIAL RESISTANCE IN Gambian NTS ISOLATES**

AMR to first line antimicrobials in NTS is present in The Gambia with patterns showing remarkable difference among various serovars, diseases and regions. MDR among S. Typhimurium and other serovars is generally low overall in sharp contrast to reports from other parts of sSA were resistance to first line antimicrobials such as ampicillin, tetracycline and chloramphenicol can exceed 70% in invasive Salmonella (Gordon & Graham, 2008; Kariuki & Revathi, 2006; Labi & Obeng-Nkrumah, 2014). Remarkably, studies in The Gambia have shown that resistance to these first line drugs have been consistently high over the years confined within invasive S. Enteritidis serovars from the eastern region of the country (Hill & Onyeama, 2007; Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015).

In one of our previous studies, in which isolates were collected from across the country and phenotypically analysed, resistance was found to be restricted to serovar. Although resistance was low for ciprofloxacin and chloramphenicol at 2% and 4% respectively, it was higher for trimethoprim-sulfamethoxazole, tetracycline and ampicillin (Kwambana-Adams & Darboe, 2015). Uniquely, up to 40% of S. Enteritidis were MDR which was strongly correlated with serovar and geographical location. In addition, a previous study from the eastern region showed 86% of invasive serovars were S. Enteritidis with high MDR to first line antimicrobials seen more in S. Enteritidis than other serovars (Ikumapayi & Antonio, 2007). The diversity of AMR between serovars and regions highlights the significance for appropriate intervention to effectively combat antimicrobial resistance. Targeting resistance in S. Enteritidis may delay emergence in other serovars. These findings are consistent with other studies that show NTS serovar differences in different geographical locations within the same country (Carroll & Wiedmann, 2017; Kariuki & Oundo, 2000).

**PROBLEM STATEMENT**
Invasive NTS is one of the leading causes of invasive bacterial disease in The Gambia including in patients with sickle cell disease. *S. Typhimurium* and *S. Enteritidis* are responsible for most cases with regional variations and endemic MDR reported further exacerbating the problem. The sub-regional emergence of MDR lineages in specific serovars implicated in invasive disease warrants investigation to determine if they are circulating in the country.

**JUSTIFICATION:**
Regional laboratory based epidemiological surveillance is critical to monitor circulating serovars as there is great geographical serovar diversity across the globe, understanding this is critical in management of patients. The incidence of iNTS and the geographic distribution is highly variable in sSA with an annual incidence of 175-388 cases per 100000 in children and 2000-7500 cases per 100000 for HIV infected adults (Feasey & Dougan, 2012). Nonetheless, there is paucity of data in many regions especially West Africa due to lack of accurate diagnostic facilities and reporting. Hence, the two virulent lineages of *S. Typhimurium* ST313 and *S. Enteritidis* West and Central/Eastern Africa clades may be underestimated and therefore, the need for advances in surveillance and monitoring iNTS epidemiological trends is paramount in the sub-region. In addition, it is important for genomic and epidemiological data be gathered across the African continent in order to further understand the genetic diversity of this important pathogen. These data are important to give insight into the genotypes present and inform therapeutic decision as infection with these lineages result in treatment failure. Thus, expansion into on global surveillance is needed to galvanise funding and political will for research and vaccine development. Furthermore, it will provide crucial information to inform policy on antimicrobial prescribing. This study therefore sought out to investigate the phenotypic and genomic diversity among NTS serovars and fill in a knowledge gap around NTS isolates circulating in The Gambia, West Africa.

In conclusion of this chapter, the changing pattern of disease presentation of the two virulent lineages of *S. Typhimurium* ST313 and *S. Enteritidis* West and Central/Eastern Africa clades may be underestimated due to lack of appropriate diagnostics. The need for advances in surveillance and monitoring iNTS epidemiological trends is of paramount importance in this sub-region. The spread of MDR iNTS can pose a threat to the effective management of salmonellosis. In addition, it is also important for genomic and epidemiological data to be gathered across the African continent to further understand the genetic diversity and epidemiology of this important pathogen.
**HYPOTHESIS:**

Virulent *Salmonella enterica* lineages causing invasive diseases in sub-Saharan Africa have emerged in The Gambia.

**OVERALL AIMS**

The study aimed to characterise NTS, describe the serovar prevalence, resistance patterns and genetic diversity of NTS associated with disease in The Gambia.

**SPECIFIC OBJECTIVES:**

1. To characterise NTS causing human disease in The Gambia including antimicrobial profile and serovar diversity using WGS reads and bioinformatic *in silico* analysis using web-based tools.
2. To determine if virulent isolates of *S. Typhimurium* and *S. Enteritidis* lineages described in sSA have emerged in The Gambia by aligning with reference appropriate reference and publicly availability genomes.
3. Use phylogenetic tree to analyse and interpret relationship among the isolates and in relation to other isolates from the sub-region.
CHAPTER 2. MATERIALS AND METHODS

STUDY SETTING AND POPULATION

The study was conducted in two locations over a period of two decades, the eastern and western regions (Basse and Fajara respectively), both served by clinics of the Medical Research Council Unit at LSHTM in The Gambia (MRCG@LSHTM). The Eastern region is known as the Upper River Region located, 373 km from the capital Banjul (Figure 5). Basse is its regional capital and regional headquarters of one of the eight Local Government Administrative Areas. It is an urban settlement surrounded by mainly rural communities with an estimated population of 200,000 people, commercial centre and a busy economic hub serving as transit route for passengers and goods to eastern Senegal, Mali and Guinea Conakry. The Western region is composed of the Greater Banjul Area within the Kanifing Local Administrative Area, 14 km from the capital city Banjul and part of the West Coast region. It is a densely populated urban settlement with a population of over 1 million people including the capital city, Banjul (Figure 4). The climate of The Gambia is sub-tropical with a warm rainy season between June and October during which most malaria transmission occurs, peaking in October subsiding in December (Ceesay & Casals-Pascual, 2010). However, malaria control interventions have resulted in a substantial decline observed in recent years coinciding with decline in NTS (Ceesay & Casals-Pascual, 2010; G. Mackenzie & Ceesay, 2010). Malnutrition remains a problem with the prevalence of underweight, stunting and wasting among children under-5 years estimated at 16.4%, 25.0% and 4.3% respectively; HIV prevalence among adults aged 15 – 49 years was estimated at 2.1% in 2015 (UNICEF, WHO, 2013).

Figure 5. Map of The Gambia (inset map of Africa showing the geographic position of The Gambia within red box) showing the western (blue box) and eastern (burgundy box) regions.
The MRCG hospitals in both regions provide primary and secondary-level care to sick individuals from the surrounding population with complicated cases referred to the main tertiary government hospitals. MRCG Basse and Fajara sites are the only health facilities in The Gambia where diagnostic microbiological cultures are routinely carried out on patients with suspected bacterial infections. In Fajara (western region), blood and cerebrospinal fluid (CSF) samples are routinely collected for bacterial culture from patients presenting with suspected sepsis and treated empirically with ampicillin and gentamicin, while those with suspected meningitis with ceftriaxone prior to laboratory confirmation. Stool samples are sent for bacterial culture for those with suspected gastroenteritis. In the Basse site (eastern region), patients’ samples sent for routine testing are recruited research study participants with clinical disease from surrounding health facilities, using similar methods to those used for non-research patients.

EThICAL APPROVAL:
This study was reviewed and approved by the Scientific Coordinating Committee of the MRCG at LSHTM Appendix I as well as the Joint Gambia Government/MRCG Joint Ethics Committee in 2016 Appendix II (SCC1498). The STROME-ID recommendation for reporting molecular epidemiological observational studies of infectious diseases was followed.

BACTERIAL ISOLATES:
The study evaluated 100 clinical NTS from 93 patients admitted to hospital with suspected sepsis (68/100), gastroenteritis (26/100), or other focal infections (6/100) from both the eastern and western regions of The Gambia. All NTS from patients visiting the clinic in the eastern region (20) were isolated in 2001 from 18 patients, and those from the western region (80) were isolated between 2006 to 2018 from 75 patients (figure 5). Isolates were stored in 15% (v/v) glycerol broth at -70°C. Isolates from the Western region were from patients of all age groups suspected and diagnosed with salmonellosis such blood cultures, stools, pus, and urine between 2005 to 2018. Those from the eastern region was mainly from blood cultures of patients mainly under 15 years in 2001.

MICROBIOLOGICAL PROCEDURES
The isolates were cultured on MacConkey agar overnight at 37°C in the Clinical Microbiology Laboratory of the MRCG@LSHTM. The laboratory is accredited to Good Clinical Laboratory Practice (GCLP; 2010) and ISO15189 (2015) as previously described (Kwambana-Adams & Darboe, 2015). Vials were retrieved from storage and inoculated on MacConkey agar plates and incubated overnight at 37°C. Original samples were processed at the Clinical Microbiology Laboratories in Fajara and Basse respectively. To confirm
identity and antibiogram for this study, all isolates were further sub-cultured onto Mueller-Hinton agar (MHA) and Xylose Lysine Deoxycholate (XLD) agar, then identified using API 20E bacterial identification system (BioMérieux Marcy-l’Etoile, France).

**ANTIMICROBIAL SUSCEPTIBILITY TESTING**

Antimicrobial susceptibility for amoxicillin-clavulanic, ampicillin, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulfamethoxazole-trimethoprim and tetracycline, were tested on Mueller-Hinton agar (MHA) using Kirby-Bauer method and interpreted according to the relevant Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2017).

A sterile loop was used to touch 3-5 colonies of each culture and suspended in 4 ml of normal physiological saline set at 0.5 McFarland standard using a densitometer. A sterile swab was dipped in suspension, excess fluid removed by pressing on edge of tube and streaked on MHA plates at 60°C angle in three directions. *Escherichia coli* (ATCC 25922) with known susceptibility profile was used as quality control organism with the following antimicrobial agents included using the following cut-offs (Table 1):

### Table 1. Cut-off values applied for interpreting zones sizes (mm) of the antimicrobials

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Disc concentration</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (Amp)</td>
<td>10 µg</td>
<td>≥ 17</td>
<td>14-16</td>
<td>≤ 13</td>
</tr>
<tr>
<td>Gentamicin (CN)</td>
<td>10 µg</td>
<td>≥ 15</td>
<td>13-14</td>
<td>≤ 12</td>
</tr>
<tr>
<td>Tetracycline (Te)</td>
<td>30 µg</td>
<td>≥ 15</td>
<td>12-14</td>
<td>≤ 11</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim (Sxt-Tmp)</td>
<td>25 µg</td>
<td>≥ 16</td>
<td>11-15</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Cefoxitin (FOX),</td>
<td>30 µg</td>
<td>≥ 18</td>
<td>15-17</td>
<td>≤ 14</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid (AMC)</td>
<td>30 µg</td>
<td>≥ 18</td>
<td>14-17</td>
<td>≤ 13</td>
</tr>
<tr>
<td>Chloramphenicol (C)</td>
<td>30 µg</td>
<td>≥ 18</td>
<td>13-17</td>
<td>≤ 12</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>5 µg</td>
<td>≥ 31</td>
<td>21-30</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>30 µg</td>
<td>≥ 21</td>
<td>18-20</td>
<td>≤ 17</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>30 µg</td>
<td>≥ 26</td>
<td>23-25</td>
<td>≤ 22</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>30 µg</td>
<td>≥ 23</td>
<td>20-22</td>
<td>≤ 19</td>
</tr>
</tbody>
</table>

Adapted from CLSI guidelines 2017 (CLSI, 2017)

**GENOMIC DNA EXTRACTION**

Genomic DNA was extracted and sequenced in two locations; most of the isolates (n=67) were processed at the University of Liverpool (UK) and sequenced at the Earlham Institute (UK). The remaining (n=33) were processed and sequenced at the MRCG@LSHTM. The DNA extraction protocol at the University of
Liverpool collected samples in barcoded tubes (FluidX tri-coded jacket 0.7 mL, 68-0702-11, Brooks Life Sciences) and sample processing was handled using a Biomek FXP instrument and a Biomek NXP automated liquid-handling workstation. The extraction protocol for the MRCG used the QIAamp DNA Mini kit (Qiagen, Germany) to extract DNA from 1.5 mL of an overnight culture grown in Tryptone Soy broth (TSB) from BD Oxoid (Basingstoke, United Kingdom) and incubated overnight at 37°C as per protocol (Appendix III). It was quantified using the Qubit fluorometer (ThermoFisher, Qubit dsDNA HS Assay), sheared using Covaris and multiplexed paired-end libraries prepared with the Nextera XT kit. Both sites used the 2x150 bp read protocol.

**WHOLE GENOME SEQUENCING (WGS)**

Genome sequencing was performed using the Illumina HiSeqTM 4000 (Illumina) at the University of Liverpool using an optimised method for large-scale sequencing (Perez-Sepulveda & Heavens, 2020), including the bespoke LITE (Low Input, Transposase Enabled) pipeline for library construction. Library construction for the HiSeq was carried out using the bespoke LITE (Low Input, Transposase Enabled) pipeline. The protocol at the MRCG@LSHTM utilised the Illumina MiSeq as previously described (Guo & Tay, 2019). The library construction for the MiSeq was done by indexing DNA fragments using Nextera. The digested ligated fragments were purified using AMPure XP beads (Beckman Coulter A63881) and quantified using the high sensitivity dsDNA qubit kit. Libraries were normalised to 10nM before pooling and quantified on a Qubit 3.0 instrument before running on a High Sensitivity D1000 ScreenTape (Agilent Catalogue No. 5067-5579) using the Agilent Tapestation. The pool was run at a final concentration of 10pM and 30x coverage on an Illumina Miseq instrument using Miseq V3 reagent kit 600 cycles following the Illumina recommended denaturation and loading recommendations (PhiX Control v3 Illumina Catalogue FC-110-3001). Data was uploaded to Basespace (www.basespace.illumina.com) where the raw data was converted to FASTQ files for each sample. Quality of the raw reads were assessed, using FASTQC (Wingett, 2018).

**GENOME ASSEMBLY AND IN SILICO ANALYSIS**

Sequencing reads of 300 base pairs (paired end) were generated for each sample and the quality of the reads assessed using FASTQC (v0.11.5) (Andrews, 2010) where on average, all reads had a quality Phred score (Qscore) above 30 (supplementary). Briefly, paired-end reads were trimmed in palindromic mode using Trimommtic (v0.39) (Bolger & Lohse, 2014) and assembled into contigs using SPades with default settings (v1.0.4) where short and low-coverage contigs were removed (Bankevich & Nurk, 2012). The Salmonella In Silico Typing Resource (SISTR) platform was used for to predict serovars from draft
assemblies by genoserotyping integrating DNA sequence-based typing for MLST, rMLST, and cgMLST (Yoshida & Kruczkiewicz, 2016). The SISTR platform is freely available and uses the Microbial In Silico Typing (MIST) on draft Salmonella genomes to predict serovars based on antigenic formula, core genome and phylogenetic relationship (Yoshida & Kruczkiewicz, 2016). The accuracy was increased by clustering and serovar-specific/lineage-specific gene markers as described (Zhang & Payne, 2019). The SISTR is robust in prediction of serovars with lower genome quality such as low N50 values and cgMLST330 loci. Contigs with 300bp or more were assembled. In sequence reads with lower quality genomes, we predicted the right serovar, considering concordance between the serovar antigenic formulae, serogroup MLST type and clustering on the phylogenetic tree. The assembled genomes were blasted against Resfinder 2.1 (Zankari & Hasman, 2012) using ABRicate and PlasmidFinder with a minimum nucleotide identity >98% as the cut-off to predict acquired antimicrobial resistance gene and plasmid replicons. Virulence factors database (VFDB) to screen for virulence genes with 100% identity as cut-off while the MLST genes were detected through BLAST search against all alleles in PubMLST schemes.

**Phylogenetic analysis**

Assembled contigs were annotated using PROKKA (v1.14.6). The core-genome was determined using roary (v3.13.0), taking the GFF files from PROKKA as input and run with default settings. The core-genome was aligned using mafft (v7.464) to generate a high-quality sequence alignment. IQ-TREE (v1.6.12) was used to infer a maximum likelihood phylogeny from the alignment and mapped to the S. Typhimurium LT2, S. Enteritidis 12 and 7795. The phylogenetic tree was visualised using the interactive Tree of Life (iTOL). Annotations for the tree were generated using custom python scripts. Resistance genes were found using abricate (v1.0.1) using the Resfinder database (2020-Apr-19). Publicly available data was downloaded from the European nucleotide archive (ENA) to compare the isolates the 18 S. Enteritidis stains from this study against 495 other African isolates. All sequence reads from which belonging to the taxid 149539 (S. Enteritidis) were downloaded and assembled/annotated using the same procedure as detailed above. The core genome phylogeny was constructed using the same methods as outlined above. A summary of the workflow is shown in Figure 6.
DATA MANAGEMENT AND STATISTICAL ANALYSIS

Patient clinical and laboratory data were collated from laboratory and clinical records from the western region and project database from the eastern region. These data were compiled into excel and linked with the metadata. As a retrospective study, the samples were already available and hence there was no need to perform any power calculations with an alpha value of 0.1 was considered statistically significant. The relationships variables were analysed using logistic regression with measures of association expressed in odds ratios. All data was analysed using various versions of Stata (StataCorp, https://www.stata.com/) dependent on publication period.

Figure 6: Summary of the DNA extraction and sequencing workflow
CHAPTER 3: RESULTS

ISOLATE SOURCE, ASSOCIATED DISEASE SYNDROME AND REGIONAL SEROVAR DIFFERENCES

One hundred isolates were recovered from clinical samples of 93 patients of all ages, with a median age range of 5-14 years from the eastern (n=20) and western (n=80) regions of The Gambia (Table 2).

Table 2. Baseline characteristics of Gambian non-typhoidal Salmonella disease patients from whom isolates were cultured for use in this study.

<table>
<thead>
<tr>
<th>Patient (n=93)</th>
<th>N (%)</th>
<th>eastern region</th>
<th>western region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4 years</td>
<td>42 (45.2)</td>
<td>4 (25.0)</td>
<td>38 (50.7)</td>
</tr>
<tr>
<td>5-14 years</td>
<td>21 (22.6)</td>
<td>12 (65.0)</td>
<td>9 (12.0)</td>
</tr>
<tr>
<td>≥15 years</td>
<td>26 (27.9)</td>
<td>2 (10.0)</td>
<td>24 (32.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (4.3)</td>
<td>0</td>
<td>4 (5.3)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51(54.3)</td>
<td>10 (55.6)</td>
<td>41 (54.7)</td>
</tr>
<tr>
<td>Female</td>
<td>38(41.5)</td>
<td>7 (38.9)</td>
<td>31 (41.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (4.2)</td>
<td>1 (5.5)</td>
<td>3 (4.0)</td>
</tr>
</tbody>
</table>

Seven duplicate sampling episodes during the same infection episode were considered as one and excluded from analysis to reduce bias in overrepresenting a subpopulation repeated testing (Table 3). Three patients had concurrent bacteraemia and gastroenteritis, two had bacteraemia with meningitis whilst two had bacteraemia with two sampling episodes.

Table 3. Patients with multisite NTS simultaneous infections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age range (yrs)</th>
<th>Disease</th>
<th>Serovar</th>
<th>Region</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>0-4</td>
<td>Bacteraemia</td>
<td>S. Enteritidis</td>
<td>Western</td>
<td>19/9/2017</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastroenteritis</td>
<td>S. Enteritidis</td>
<td></td>
<td>14/9/2017</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>35-39</td>
<td>Bacteraemia</td>
<td>S. Typhimurium</td>
<td>Western</td>
<td>1/1/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastroenteritis</td>
<td>S. Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>0-4</td>
<td>Bacteraemia</td>
<td>S. Virchow</td>
<td>Western</td>
<td>1/3/2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>30-34</td>
<td>Bacteraemia</td>
<td>S. Typhimurium</td>
<td>Western</td>
<td>1/1/2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastroenteritis</td>
<td>S. Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>0-4</td>
<td>Bacteraemia</td>
<td>1,6,14,25:y:1,5</td>
<td>Western</td>
<td>1/10/2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>5-9</td>
<td>Bacteraemia</td>
<td>S. Enteritidis</td>
<td>Eastern</td>
<td>1/10/2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. Enteritidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>0-4</td>
<td>Bacteraemia</td>
<td>S. Typhimurium</td>
<td>Eastern</td>
<td>1/12/2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meningitis</td>
<td>S. Typhimurium</td>
<td></td>
<td>1/12/2001</td>
</tr>
</tbody>
</table>
Isolates were recovered from 18 patients from the eastern region predominantly from invasive disease (17 blood and 2 CSF) with only 1 gastroenteritis (stool) source cases; whilst 80 isolates recovered from 75 patients in the Western region were associated with invasive disease (48 blood and 1 CSF), gastroenteritis (25 stool), and other focal non-invasive infections (5 abscesses/pus and 1 urine) (Table 4).

<table>
<thead>
<tr>
<th>Disease syndrome</th>
<th>Total</th>
<th>Eastern region (2001) n=20</th>
<th>Western region (2006-2018) n=80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Invasive disease</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td>17</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>9</td>
<td>9 (47.1)</td>
<td>0</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>8</td>
<td>6 (35.3)</td>
<td>2</td>
</tr>
<tr>
<td>S. Virchow</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other serovars#</td>
<td>3</td>
<td>3 (17.6)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Other = Abscess/pus and urine isolates
#Other serovars shown in Figure 7
Salmonella serovars other than S. Enteritidis, S. Typhimurium and S. Virchow were primarily responsible for gastroenteritis (17/23; 73.9%), whilst S. Typhimurium and S. Enteritidis were the leading cause of invasive disease. Overall, S. Typhimurium and S. Enteritidis were 15 times and nearly three times as likely to cause invasive disease than gastroenteritis, respectively (Table 5).

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Total</th>
<th>Invasive N (%)</th>
<th>Gastroenteritis N (%)</th>
<th>Others N (%)</th>
<th>Odds of Invasive vs Gastroenteritis</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>93</td>
<td>64</td>
<td>23</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>28</td>
<td>26 (30.6)</td>
<td>3 (4.3)</td>
<td>1 (16.7)</td>
<td>15.05</td>
<td>1.91; 118.48</td>
<td>0.010</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>16</td>
<td>13 (20.3)</td>
<td>3 (8.7)</td>
<td>1 (16.7)</td>
<td>2.68</td>
<td>0.52; 7.58</td>
<td>0.220</td>
</tr>
<tr>
<td>S. Virchow</td>
<td>7</td>
<td>4 (6.3)</td>
<td>3 (13.0)</td>
<td>0 (0)</td>
<td>0.44</td>
<td>0.13; 2.75</td>
<td>0.314</td>
</tr>
<tr>
<td>Other serovars*</td>
<td>42</td>
<td>21 (32.4)</td>
<td>17 (65.8)</td>
<td>4 (66.7)</td>
<td>0.17</td>
<td>0.06; 0.50</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Listed in appendix III

In terms of regional prevalence, S. Typhimurium was the leading serovar responsible for bacteraemia in the Western region and S. Enteritidis in the Eastern region (Figure 7). In addition, S. Virchow was equally an important cause of bacteraemia in the Western region as S. Enteritidis.

Figure 7: Bar chart showing the major NTS serovar prevalence by region
All *S. Typhimurium* were in eBG1 and assigned to a single sequence type, ST19 with one or two allelic variants (Table 6). All *S. Enteritidis* belonged to eBG4 assigned to ST11 and ST1925, including two isolates having single locus allelic variants. *S. Virchow* was in eBG9 and assigned to three different STs as follows: ST181, ST755 and ST841. The four *S. Hull* isolates belonged to eBG330 and assigned ST1996 with single locus variant. *S. Stanleyville* eBG79 (ST339), *S. Poona* eBG46 (ST308) and *S. Give* eBG67 (ST516) all belonged to a single sequence type.

Table 6. eBurst groups of the major Gambian NTS serovars responsible for clinical disease

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Sequence type</th>
<th>Number</th>
<th>eBG</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Enteritidis</em></td>
<td>11</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1925</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>S. Typhimurium</em></td>
<td>19</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td><em>S. Virchow</em></td>
<td>181</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>755</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>841</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**AMR GENES, AMR PHENOTYPES, PLASMID REPLICONS AND VIRULENCE GENES**

Antimicrobial resistance genes belonging to eight classes of antimicrobials were detected, plus a biocide tolerance genetic determinant. The genes encoding an aminoglycoside modifying enzyme *aac(6’)-Iaa_1* was present in all isolates, but did not have any detectable phenotypic effects (Figure 8). Other AMR genes were harboured by 16/93 (17.2%) isolates and conferred resistance to aminoglycosides (*aph_3_1b* and *aph_6_Id*; n=12), tetracyclines (*tet_A* and *tet_B*; n=9), trimethoprim (*dfra14*, *dfra7* and *dfra8*; n=8), sulfamethoxazole (*sul2* and *sul1*; n=7), ampicillin (*blaTEM-1B*; n=8), fosfomycin (*fosA7_1*; n=7), azithromycin (*mph_A*; n=3) and chloramphenicol (*catA1_1*; n=2) (Figure 8). Possession of three or more MDR genes (resistance to 3 or more antimicrobials) was found in 9/93 (9.7%) isolates of which 7/9 (77.8%) were found in *S. Enteritidis*. Only one *S. Typhimurium* and *S. Wernigerode* isolates harboured MDR in addition to the *S. Enteritidis*. All three *S. Stanleyville*, and each of *S. Grumpensis*, *S. Wernigeroid* and serovar 1.6.14.25:y:1.5, isolates harboured the *fosA7_1* resistance gene (Table 7).
Table 7. Summary of genotypic and phenotypic characteristics of serovars with resistance genes

<table>
<thead>
<tr>
<th>Isolate</th>
<th>ST</th>
<th>Sample</th>
<th>Region</th>
<th>resistance genes</th>
<th>Phenotypic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0796_06; S. Lomita</td>
<td>3039</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>1058_09; S. Stanleyville</td>
<td>339</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>1427_09; S. Stanleyville</td>
<td>339</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>4585_16; S. Stanleyville</td>
<td>339</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>1503_08; 1,6,14,25:y:1,5</td>
<td>6046</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>2460_13; S. Grumpensis</td>
<td>2060</td>
<td>Blood</td>
<td>western region</td>
<td>fosA7_1</td>
<td>Not tested</td>
</tr>
<tr>
<td>2933_14; S. Wernigerode</td>
<td>2271</td>
<td>Stool</td>
<td>western region</td>
<td>fosA7_1, blaTEM-1B_1, aph_3_lb_5, dfrA8_1, tet_B_2</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim, Tetracycline</td>
</tr>
<tr>
<td>3625_14; S. Enteritidis</td>
<td>1925</td>
<td>Urine</td>
<td>western region</td>
<td>tet_A_6</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>0081_15; S. Typhimurium</td>
<td>19</td>
<td>Blood</td>
<td>western region</td>
<td>tet_A_6, sul2_2, aph_6_id_1, aph_3_lb_5</td>
<td>Tetracycline, sulfamethoxazole-trimethoprim</td>
</tr>
<tr>
<td>0008_01; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>eastern region</td>
<td>aph_6_id_1, blaTEM-1B_1, dfrA14_5, sul2_2, aph_6_id_1</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim</td>
</tr>
<tr>
<td>1004_01; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>eastern region</td>
<td>blaTEM-1B_1, dfrA14_5, tet_A_6, sul2_2, aph_6_id_1</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim tetracycline</td>
</tr>
<tr>
<td>8078_01; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>eastern region</td>
<td>blaTEM-1B_1, dfrA14_5, tet_A_6, sul2_2, aph_6_id_1</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim tetracycline</td>
</tr>
<tr>
<td>0378_01; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>eastern region</td>
<td>blaTEM-1B_1, dfrA14_5, tet_A_6, sul2_2, aph_6_id_1</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim tetracycline</td>
</tr>
<tr>
<td>0527_01; S. Enteritidis</td>
<td>11</td>
<td>Stool</td>
<td>eastern region</td>
<td>blaTEM-1B_1, dfrA14_5, tet_A_6, sul2_2, aph_6_id_1</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim tetracycline</td>
</tr>
<tr>
<td>4025_16; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>western region</td>
<td>blaTEM-1B_1, dfrA7_5, catA1_1, sul1_5, tet_B_2, aph_3_lb_5, aph_6_id_1, mph_A_2</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim tetracycline chloramphenicol</td>
</tr>
<tr>
<td>4030_15; S. Enteritidis</td>
<td>11</td>
<td>Blood</td>
<td>western region</td>
<td>blaTEM-1B_1, dfrA7_5, catA1_1, sul1_5, tet_B_2, aph_3_lb_5, mph_A_2, mph_A_2</td>
<td>Ampicillin, sulfamethoxazole-trimethoprim, tetracycline chloramphenicol</td>
</tr>
</tbody>
</table>
Figure 8: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of the samples. 
*Salmonella* serovar, disease type, source region within Gambia, and year of isolation have been labelled for each isolate. The tree was built using maximum-likelihood methods implemented in IQ-TREE followed by mid-point rooting. The presence of phenotypic (solid-coloured squares) and genotypic (solid black squares) resistance genes is shown. Bar, 0.001 changes per substitution site. Most of the multi-drug resistant isolates can be found in the *S. Enteritidis* clades sourced from the eastern region of the Gambia. Amp: ampicillin; SXT: sulfamethoxazole; Te: tetracycline; C: chloramphenicol.
Phenotypic resistance was observed for tetracycline, ampicillin, sulfamethoxazole-trimethoprim, and chloramphenicol in 9/93 (9.7%) isolates, correlating with the presence of resistance genes, except for streptomycin, fosfomycin and azithromycin that were not phenotypically tested due to lack of clinical relevance or appropriate testing method (Figure 8). The odds of resistance to ampicillin, sulfamethoxazole-trimethoprim and tetracycline were respectively 59, 23 and 29 times more likely for S. Enteritidis than all other serovars combined (Table 8). No resistance to gentamicin was observed phenotypically despite the presence of two aminoglycoside resistance genes (aph_3_Ib and aph_6_Id), which only confer resistance to streptomycin.

Table 8. Odds ratio of S. Enteritidis MDR against all NTS serovars

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>S. Enteritidis n=16</th>
<th>Other serovars n=67</th>
<th>Odds of S. Enteritidis vs all serovars</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp</td>
<td>7</td>
<td>1</td>
<td>59.1</td>
<td>6.51;536.85</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Sxt</td>
<td>6</td>
<td>2</td>
<td>22.5</td>
<td>3.61;127.05</td>
<td>0.004</td>
</tr>
<tr>
<td>Te</td>
<td>7</td>
<td>2</td>
<td>29.2</td>
<td>5.24;162.37</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Nineteen different plasmid replicons were detected in 61/93 (65.6%) isolates; 7 isolates harboured one plasmid replicon, 35 harboured two, 15 harboured three and 4 harboured four plasmid replicons. The most common plasmids were IncFII (n=50) and IncFIB (n=45), harboured by all S. Typhimurium and all but the two chloramphenicol-resistant S. Enteritidis (Table 9). The S. Typhimurium isolates harboured more plasmids than the S. Enteritidis.

Table 9. Summary of plasmid replicons and serovar harbouring them in Gambian non-typhoidal Salmonella isolates.

<table>
<thead>
<tr>
<th>Plasmid Type</th>
<th>Total</th>
<th>S. Enteritidis n=16</th>
<th>S. Typhimurium n=28</th>
<th>S. Virchow n=7</th>
<th>Other serovars n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td>IncFII_S__1</td>
<td>50</td>
<td>16</td>
<td>31</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>IncFIB_S__1</td>
<td>45</td>
<td>16</td>
<td>31</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IncI1_1_Alcro</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IncN_1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IncX1_1</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IncFIB_pPHS1__1_pPHS1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IncFII_SARC14__1_SARC14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IncFII_p14_1-p14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IncL/M_pOXA-48_1_pXA-48</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>pSL483_1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ColRNAI_1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Col_MG828_1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IncFII_pB171__1_pB171</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IncI2_1_Delta</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IncX1_4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IncX3_1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>repUS21__rep_pWBG764</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IncFII_pRSB107__1_pRSB107</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>pENTAS02_1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The IncN_1 plasmid was associated with MDR including azithromycin resistance and was only found in S. Enteritidis from the eastern region (Figure 9b). The IncI1_1_Alcro was harboured by the two chloramphenicol MDR S. Enteritidis isolates from the western region and the susceptible isolates from the eastern region (Figure 9b).

Notably, no plasmid replicons were detected in S. Bradford, S. Hull, S. Stanleyville, S. Rubislaw, S. Vinohrady and 1,4,12,27:g,m:1,2 serovars.
The presence of virulence genes varied across the Gambian NTS serovars. The highest average number of virulence genes was seen for *S. Typhimurium* with the notable absence of *entA, entB, entE, faeC, faeD, faeE, fepC and fepG* in all Gambian NTS serovars. In addition, the *cdtB, ssPH1 and shdA* were absent for *S. Typhimurium*. Similarly, virulence genes for *S. Enteritidis* isolates were all missing the above-mentioned genes as well as *gogB, grvA, sinH, slrP, and sseK2*, the *spaS* gene was only present in the *S. Enteritidis* outlier global clade whilst a notable absence of the *ssaG* and *ssaO* virulence genes were observed for the global *S. Enteritidis* epidemic clade in this setting.

**Phylogenetic Analysis**

The assembled contigs using core genome analysis showed that the isolates were clustered into respective serovars (Figure 8) with *S. Enteritidis* harbouring most of the resistant genes. The *S. Enteritidis* isolates were further put within the wider regional context and compared to 495 African *S. Enteritidis* isolates available from the European Nucleotide Archive (ENA). This analysis revealed considerable genetic diversity confirming three clades: the global epidemic clade (n=3) and global outlier clade (n=2) known to cause human gastroenteritis and the West African clade (n=11) known to cause invasive diseases carrying the *catA1* gene (Feasey & Hadfield, 2016) (Figure 9a). All *S. Enteritidis* isolates from the eastern region (n=8) and from the western region (n=3) fell within the West African clade, clustering closely with *S. Enteritidis* isolates from Ghana, Guinea and Mali. Among the West African clade, 7/11 (66.6%) were MDR (5 from the Eastern region and two from the Western region) and the remaining were pan susceptible isolates. Among all isolates within the West African clade from the sub-region in the study, azithromycin resistance *mph_A_2* gene was only harboured by isolates from the Eastern region (Figure 9b). The median SNP distance for *S. Enteritidis* was 459 (Figure 10) and *S. Typhimurium* ST19 demonstrated clonality with median SNP distance of 180 (Figure 11). *S. Enteritidis* isolates belonging to the global outlier clade were isolated from blood (n=1) and stool (n=1), whilst those within the global epidemic isolates were isolated from blood (n=2) and urine (n=1).
Figure 9a: Maximum likelihood genome phylogeny of Salmonella Enteritidis (NCBI: txid149539) from across Africa. The phylogeny of S. Enteritidis from the African region. The maximum-likelihood tree was constructed using iqtree with the scale bar representing genetic distance. The tree was rooted using the mid-point rooting technique. The majority of the isolates from this study are placed within the West African clade. Samples from this study have been labelled on the outer ring.
Figure 9b: Phylogenetic tree showing presence and absence of antimicrobial resistance-associated genes of S. Enteritidis within the West African clade. The tree was built by applying maximum likelihood phylogenetic reconstruction using method implemented by iqtree followed by mid-point rooting, with a scale bar showing the maximum likelihood genetic distance estimated by iqtree. Each branch is labelled with either isolate name (this study) or accession number (reference isolates), followed by country of isolation. Presence (filled squares) or absence (empty squares) of antimicrobial resistance genes and plasmid replicons are displayed for each isolate. A scale bar is present showing the maximum likelihood genetic distance estimated by iqtree.
Figure 10: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of S. Typhimurium isolates showing plasmids present within each isolate using the S. Enteritidis P125109 reference genome showing disease type, source region within Gambia, for each isolate. The tree was built using maximum-likelihood methods implemented in IQ-TREE followed by mid-point rooting.
Figure 11: Phylogenetic tree reconstructed with IQ-TREE using the core-genome of S. Typhimurium isolates showing plasmids present within each isolate using the S. Typhimurium LT2 reference genome showing disease type, source region within Gambia, for each isolate. The tree was built using maximum-likelihood methods implemented in IQ-TREE followed by mid-point rooting.
CHAPTER 4: DISCUSSION

CIRCULATING SEROVAR DIVERSITY

This study utilised WGS and phylogenetic analysis to confirm the circulation of NTS serovars including presence of virulent MDR epidemic S. Enteritidis West African clade but did not find S. Typhimurium ST313. In addition, the phylogenetic analysis has shown two additional S. Enteritidis clades; the Global Epidemic and outlier clades associated with poultry and egg epidemics (Aldrich & Hartman, 2019; Feasey & Hadfield, 2016). The epidemic S. Enteritidis West African clade was mainly confined in the eastern region as far as 2001 which also has a slightly higher rate of HIV (Schim Van Der Loeff & Sarge-Njie, 2003) and malaria prevalence (Mwesigwa & Okebe, 2015). Studies have shown this clade to be associated with high mortality, harbouring MDR and exhibiting genome degradation, thus, adapting to an invasive lifestyle (Aldrich & Hartman, 2019; Feasey & Hadfield, 2016). Host factors such as immune suppression, age and malaria infection are possibly contributing to these invasive virulent lineages (Feasey & Hadfield, 2016; Okoro & Kingsley, 2012); however, these host factors were not assessed in this study. These findings have important implications for accurate antimicrobial prescribing and regional management of NTS disease. One-third of the West African clade S. Enteritidis in this study did not harbour resistance genes and were phenotypically susceptible as opposed to the high MDR in this clade reported by other studies (Aldrich & Hartman, 2019; Feasey & Hadfield, 2016). This could be an interesting adaptation mechanism that requires investigation in the evolution for S. Enteritidis as described for S. Typhimurium susceptible lineages (Pulford & Perez-sepulveda, 2021).

The other two global clades associated with poultry and egg production were found confined in the western region of the country. The isolates from the eastern region clustered closely with isolates from Mali and Guinea providing evidence of subregional dissemination and circulation of virulent phenotypes. This circulating West African clade is associated with high mortality, harbouring MDR and exhibit genome degradation facilitating an invasive lifestyle (Aldrich & Hartman, 2019; Feasey & Hadfield, 2016). Although MDR is an important prerequisite for this clade, fascinatingly, almost half of the S. Enteritidis in this study within the epidemic West African clade did not harbour any resistant gene. This could be an interesting adaptation mechanism seen in S. Typhimurium susceptible lineages and requires investigation in the evolution for S. Enteritidis (Pulford & Perez-sepulveda, 2021). This therefore warrants further epidemiological investigations and surveillance as the MDR has important implications in treatment guidelines (Feasey & Hadfield, 2016). The S. Enteritidis isolates sequence
types (ST11 and ST1925) within this study were within eBurst Group 4 inferring evolutionary descent within a cluster.

Remarkably, phylogenetic analysis found only *S*. Typhimurium ST19 causing both invasive diseases and gastroenteritis within this study as opposed to ST313 virulent lineage, endemic in other parts of sSA (Branchu & Bawn, 2018; Okoro & Kingsley, 2012). A plausible explanation is that the sequence type ST313 is associated with HIV infection which has a low prevalence in The Gambia (National AIDS Secretariat, 2015). However, recent data emerging from the eastern region with higher HIV prevalence has confirmed the presence of ST313 lineage in this part of the country (Kanteh & Sesay, 2021). Furthermore, the GEMS population-based case-control study of acute moderate to severe diarrhoea among children under 5 years determined that NTS is prevalent, although not a major cause of gastroenteritis in Africa (Kasumba & Pulford, 2021; Kotloff & Nataro, 2013). Molecular analysis of the NTS confirmed the presence of ST313 asymptomatic carriage and in diarrhoeal patients further highlighting that sequence type is not only associated with invasive disease and demonstrating anthroponotic transmission (Kasumba & Pulford, 2021). This difference in pathogenesis warrants further comparative genomics and epidemiological investigations into this important cause of invasive disease in this setting. This study also further provides evidence that although, *S*. Typhimurium ST19 remains a leading cause of invasive disease in addition to the virulent lineages of *S*. Enteritidis. Although *S*. Typhimurium ST19 still remains associated with invasive disease in the sub-region, it is usually more susceptible to antimicrobials (Pulford & Perez-sepulveda, 2021).

Although being the 3rd most prevalent serovar in this study, *S*. Virchow was exclusively confined within the western region causing both invasive diseases and gastroenteritis across all age groups. Although the reason for this is beyond the scope of this study, a previous molecular study also showed less serovar diversity in serovar prevalence within invasive disease in the eastern region (Ikumapayi & Antonio, 2007). However, *S*. Virchow a predominant serovar mainly in Asia, Europe and Oceania (Hendriksen & Vieira, 2011) is increasingly becoming a serovar responsible for disease in The Gambia with three sequence types (ST181, ST755 and ST841) found all belonging to eBurst Group 9.

Two-thirds of serovars responsible for gastroenteritis were serovars other than *S*. Typhimurium and *S*. Enteritidis as opposed to other parts of the world where these two serovars account for the highest burden (Hendriksen & Vieira, 2011). The difference in the number of isolates of serovars causing gastroenteritis as opposed to iNTS may be due to sampling bias, being patients presenting to hospital
with obvious clinical syndromes necessitating laboratory investigation. Notwithstanding, other serovars were still an important cause of invasive disease responsible for up to 30% of infections. To further put this into context, a recent population-based study found serovars other than *S. Enteritidis* and *S. Typhimurium* that were also important causes of invasive NTS in the eastern region (Kanteh & Sesay, 2021). *S. Dublin* was confirmed as the most common serovar as opposed to *S. Virchow* found in this study for the western region. In addition, the majority of serovars in this study belong to the *S. enterica* subspecies *enterica* clade B which are specialised and adapted to the gastrointestinal niche, hence likely to cause such infections (den Bakker & Moreno Switt, 2011). Similarly, the enteric study nearly a decade ago by colleagues in the eastern region, focused on transmission dynamics, found no *S. Enteritidis* or *S. Typhimurium* in NTS causing gastroenteritis with *S. Colindale* being the most common serovar from humans (Dione & Ikumapayi, 2011). This study on children with gastroenteritis living in close proximity with animals found similar clade B serovars among the NTS isolated and further discounted zoonotic transmission (Dione & Ikumapayi, 2011), in contrast to what was observed in Kenya and Malawi with zoonotic transmission from pigs (Wilson & Pulford, 2020). In addition, another Kenyan study found close relatedness among serovars isolated from blood and stool of patient and asymptomatic carriers, underscoring a common source (Kariuki & Mbae, 2020). It is therefore plausible that NTS serovars in The Gambia are site specific, i.e., those causing enteric diseases are different from those causing invasive diseases. More epidemiological studies are needed to validate this hypothesis. The great diversity in serovars causing invasive disease and gastroenteritis suggests NTS gastroenteritis may not be a predisposition to invasive NTS in The Gambia. Although a huge gap exists regarding transmission dynamics of NTS in sSA (Gordon, 2012), more insights are needed to understand the relationship between NTS gastroenteritis and iNTS diseases.

In addition, the past two decades have revealed geographical serovar diversity between the two regions, indicating a possible regional specific epidemiological pattern of NTS disease in The Gambia. However, the time difference in the sampling between the two regions may confound the difference in location and warrant further investigation. Nonetheless, previous phenotypic studies have highlighted these serovar differences (Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015). The changing disease pattern of NTS associated with specific lineages of *S. Typhimurium* and *S. Enteritidis*, remains a major concern in sSA and warrants for stringent surveillance and containment measures (Feasey & Hadfield, 2016; Kingsley & Msefula, 2009; Mahon & Fields, 2016; Uche et al., 2017). In line with this, three cases of *Salmonella* bacterial meningitis were included in this study, all of which were found in
paediatric patients under 10 years old. This suggests that iNTS may be a leading cause of bacteraemia in The Gambia. Similarly, NTS meningitis, though rare, has been reported elsewhere in Africa, and is often associated with high case fatality (Keddy & Sooka, 2015; Molyneux & Mankhambo, 2009). Therefore, NTS needs to be considered in the differential diagnosis of bacterial meningitis following post-vaccine declines in the prevalence of Hib, Neisseria meningitidis and pneumococcal meningitis (G. A. Mackenzie & Hill, 2016; Zaman & Howie, 2020).

Although serovar designations do not necessarily infer genetic relatedness (Achtman & Wain, 2012) in Salmonella, our NTS serovars were in the same eBG and clustered closely together in the phylogenetic tree confirming close relationship. In addition, the sequence types of the major serovars were mainly confined within one or two STs indicating genetic relatedness. Achtman et al., have recommended the need to replace serotyping with MLST or equivalents (Achtman & Wain, 2012). Despite the observed differences in proportions S. Typhimurium was the most prevalent serovar in Western region whilst S. Enteritidis was predominant in the Eastern region. Temporal shift is highly discounted as serological analysis of isolates from studies around the same time period in both regions highlight this difference (Dione & Ikumapayi, 2011; Ikumapayi & Antonio, 2007; Kwambana-Adams & Darboe, 2015).

**ANTIMICROBIAL RESISTANCE GENES AND PLASMID REPLICONS**

There were varied resistance genes prevailing among NTS serovars including resistance genes coding for streptomycin, azithromycin and fosfomycin. The emergence of chloramphenicol and azithromycin resistance warrants monitoring as azithromycin is drug of choice in invasive salmonellosis. Importantly, azithromycin resistance has been described for a human host adapted MDR sub-lineage of S. Typhimurium ST313 (Van Puyvelde & Pickard, 2019). MDR was also found to be correlated with serovar, plasmid replicon and geographical location. Although the use of WGS for AMR prediction has revolutionised surveillance, its widespread use remain controversial due to lack of sufficient data for most bacterial species (Ellington & Ekelund, 2017; Su & Satola, 2019), high concordance with phenotypic AST has been documented in several studies for NTS (McDermott & Tyson, 2016; Monk, 2019; Neuert & Nair, 2018; Pornsukarom & Van Vliet, 2018). Additionally, MDR for first-line antimicrobials such as ampicillin, sulfamethoxazole-trimethoprim, tetracycline, and chloramphenicol were confined within S. Enteritidis. This study did not phenotypically test streptomycin susceptibility which lacks clinical breakpoints, and it is not used in the treatment of infections. In addition, the streptomycin resistance genes were frequently found to lack expression (Springer & Kidan, 2001). Although no fluoroquinolone or cephalosporin resistance was identified, implying these drugs might still
be effective in The Gambia, the emergence of azithromycin resistance gene mph A requires further monitoring as a recommended drug of choice for management of iNTS (Gomes & Martínez-Puchol, 2017). The aminoglycoside resistance gene aac (6′)-Iaa was present in all serovars including pan-susceptible isolates warranting surveillance to ensure emerging trends are identified promptly for appropriate intervention.

*S. Typhimurium* serovars causing MDR disease circulating in sSA was notably absent. Geographic differences seen in AMR may reveal differences in selective pressure and ecological factors thus suggesting need for location specific control measures. The diversity of AMR between serovars and geographic regions highlights the need for real-time surveillance as well as region-appropriate interventions to effectively combat AMR in The Gambia. Consequently, this study highlights the potential of using genomic-based AMR prediction to monitor AMR determinants for emerging resistance. While the development of AMR has been mainly attributed to antimicrobial misuse in humans and animals, evidence has shown that environmental factors such as poor sanitation, hygiene and access to clean water may be equally responsible for driving resistance in LMIC (Afema & Byarugaba, 2016; Laxminarayan & Duse, 2013). A study underscored distinct factors such as use of antimicrobials in food producing animals as contributing to emergence and dispersal of AMR in humans (Carroll & Wiedmann, 2017). The cryptic aminoglycoside resistance gene aac(6′)-Iaa known to have no evolutionary potential to increase resistance to aminoglycosides was found in all serovars. Surveillance is however warranted for other AMR determinants such the presence of biocide tolerance as these are strongly collaborated with environmental and transmission through the food chain in Salmonella and linked to MDR (Fernández Márquez & Burgos, 2017; Ortega Morente & Fernández-Fuentes, 2013). The findings of this study are consistent with other studies that showed NTS serovar differences in geographical locations within the same country (Carroll & Wiedmann, 2017; Kariuki & Oundo, 2000).

The *IncN* type plasmid replicon was strongly associated with resistance and was found only within multi-drug resistant *S. Enteritidis*, thus requiring closer surveillance. This plasmid is associated with dissemination of AMR with high potential of spread (García-Fernández & Villa, 2011). The use of short reads for analysis of plasmids remains a challenge due to long repeat sequences (Arredondo-Alonso & Willems, 2017). However, the short read replicon predictions by PlasmidFinder uses previously assembled contigs from a plasmid in databases to improve accuracy (Carattoli & Zankari, 2014). The *IncII* plasmid carries *spv* locus and multi-drug encoding cassette is worrisome as this may be
disseminated via horizontal gene transfer. The *IncF* plasmid replicons possess non-conjugative virulence plasmids.

Many virulence genes were found among our NTS isolates, however, the identification of virulence factors coding for specific phenotypic traits can be challenging. Some virulence genes have been found to code for different virulence traits among different serovars (Van Asten & Van Dijk, 2005). Notwithstanding, the pathogenic success of NTS serovars is directly linked to their plethora of virulence factors aided by host susceptibility, serovar fitness, infectious dose and AMR (Cheng & Eade, 2019).

In addition, the *cdtB* gene, rarely reported in NTS and considered to contribute towards pathogenicity, was absent in the major serovars responsible for clinical disease but found in serovars such as *S. Bradford*, *S. Give* and *S. Poona*. Further studies are needed to understand the clinical implications of these virulence genes.

There are several limitations in this study. First, it is a retrospective study with the isolates collected at different time points, with a lag of up to 18 years between the two different regions, which may lead to missing temporal differences. In addition, clinical history, such as disease severity and treatment data, were not collected or assessed, which may have provided additional information regarding the virulence and drug resistance characteristics of the NTS isolates analysed. No population denominator and hence true population prevalence in some of the studies may be possibly underestimated as not all cases come to hospital. In addition, many patients do not report to hospitals and even when they attend, treatment is usually empiric due to lack of appropriate diagnostic facilities. However, the higher AMR prevalence of *S. Enteritidis* in the Eastern region as early as 2001 compared to the more recent Western region proves the point that AMR emerged a lot earlier and is likely more prevalent in the Eastern region.

Second, relatively few isolates were analysed from only two regions due to limited microbiology capacity resulting in wide confidence intervals. Therefore, this study may not reflect the entirety of isolates and lineages of the NTS in The Gambia. Due to the retrospective nature of the study, no consistent procedures for investigation of patients and time points were available and hence different levels of bias in sample collection like skin preparation, sample volume, different time points and prior antimicrobial use is lacking. There is a lack of standardized case enrolment criteria, with the eastern region isolates primarily from patients enrolled in study projects. However, the current validity of the data reported here is supported by recent epidemiological data confirming that multi-drug resistant *S. Enteritidis* remains the most prevalent serovar in the Eastern region. Blood cultures are not sensitive in detecting bacteraemia as prior antimicrobial use and right blood volume affect sensitivity. The
serological serotyping was limited only to three serovars resulting in a large proportion of untyped NTS. Although the retrospective nature of this study resulted in major limitations, this work demonstrated that it can be harnessed for disease surveillance in monitoring local trends for effective to improve patient management and public health intervention.
This study used WGS to investigate NTS serovars and assess their phylogenetic relatedness in The Gambia. Importantly, it has confirmed the presence of the invasive epidemic \( S. \) Enteritidis West African clade in The Gambia, mainly in the Eastern region of the country. The genotypic analysis confirmed the presence of resistance including chloramphenicol and azithromycin resistance genes, but no fluoroquinolone resistance. The majority of patients that had isolates from the West African clade were mainly from the eastern region which has a slightly higher rate of HIV (Schim Van Der Loeff & Sarge-Njie, 2003) and malaria prevalence (Mwesigwa & Okebe, 2015). This warrants further epidemiological investigations and surveillance as it has important implication in treatment. The implications of NTS as a zoonotic foodborne disease is well established globally with virulence dependent on both host factors and serovar (Afema, 2016; Eng & Pusparajah, 2015; Fàbrega & Vila, 2013; Knodler & Elfenbein, 2019; Wilson & Pulford, 2020). However, differences in transmission dynamics have been noted for sSA and high income settings with co-morbidity an important factor (Crump & Heyderman, 2015). Notwithstanding evidence for a zoonotic transmission route remains to be established in sSA (Dione & Ikumapayi, 2011; Kariuki & Revathi, 2006). Importantly the anthropogenic transmission route has gained significance in sSA as asymptomatic carriage remains a major reservoir of disease (Kariuki & Revathi, 2006; Kasumba & Pulford, 2021). In addition, phylogenetic analysis among isolates from blood and stool in both cases and control in studies across the subregion revealed high relatedness suggesting a common source (Kariuki & Mbae, 2020; Kasumba & Pulford, 2021). This further highlights environmental factors as important routes of transmission (Afema & Byarugaba, 2016) warranting surveillance in The Gambia as this is yet to be established.

The high morbidity and mortality associated with iNTS among vulnerable populations in sSA due to unavailability of effective antimicrobials merits introducing vaccines as control strategies targeted to vulnerable populations. Although NTS serovars are diverse making vaccine development a challenge including lack of cross protection among different serovars (Gayet & Bioley, 2017; MacLennan & Martin, 2014), O-antigen-based conjugate vaccines have demonstrated protection within same serogroups for the predominant serovars (Tennant & MacLennan, 2016) and could be explored as a plausible preventive strategy for iNTS disease. In addition, the major serovars responsible could be targeted for vaccine development to reduce burden. Despite efforts made in the development of NTS vaccines, suffice to say, they are yet to be licenced and rolled out for use (MacLennan & Martin, 2014).
The results of this study highlight how the significance of WGS-based MDR prediction can be used to monitor MDR determinants for potential future emergence of resistance. While the data confirmed MDR was found to be correlated with serovar type, plasmid replicon and geographical location, surveillance is key in combating MDR. Our study did not phenotypically test streptomycin susceptibility, which lacks clinical breakpoints, and it is not used in the treatment of infections. In addition, the streptomycin resistance genes were frequently found to lack expression (Springer & Kidan, 2001). Although the development of MDR has been mainly attributed to misuse in humans and animals, evidence has shown that environmental factors such as poor sanitation and access to clean water may equally be responsible in LMIC (Afema & Byarugaba, 2016; Laxminarayan & Duse, 2013). We have demonstrated that monitoring local trends in MDR for S. Enteritidis is warranted as exchange of plasmids carrying resistance between closely related species is common. More research is needed in the epidemiology of NTS and dissemination of resistance for the management of febrile illness.

In conclusion, this study has confirmed the emergence of multi-drug resistant epidemic S. Enteritidis West African clade in The Gambia, confined mainly in the Eastern region of the country. These findings have important implications for antimicrobial prescription policies and regional surveillance of NTS disease. This study has demonstrated that a robust genomic epidemiological surveillance of NTS by WGS can be instrumental in generating critical knowledge and timely information for better disease management and prevention.

**Further perspectives:**

The importance of *Salmonella* as a pathogen causing serious infections and the emergence of MDR isolates warrant structured disease monitoring and surveillance across the sub-Saharan African continent and in The Gambia in particular. Well-designed prospective epidemiological investigations are needed to assess the burden of iNTS disease in The Gambia. Stringent control measures need to be implemented from the ‘farm-to fork’ to reduce infection. This warrants quintessential collaborative One Health approach to increase awareness and education for improved behavioural interventions that could reduce disease burden. This is important to validate regional serovar and resistance differences. With the emergence of invasive MDR clades in S. Enteritidis, further investigation into the transmission dynamics of iNTS is warranted. The need for improved microbiologic diagnostics facilities for appropriate resistance testing is paramount. The incidence and mortality rates among iNTS continue to
decline and lower than other parts of sSA corresponding with substantial malarial decline. However, it remains a leading cause of bacteraemia across all age groups. Patients presenting with multiple episodes need to be further investigated to confirm if they are re-infections or recrudescent. In addition, sources and transmission pathways need to be evaluated for effective prevention and control strategies. Consequently, surveillance of zoonotic and environmental transmission especially the investigation of slaughterhouses and imported food is also warranted. Finally, further evidence for human versus animal reservoir of iNTS need to be established in our setting as data from other African studies begin to support human-to-human transmission.
REFERENCES


APPENDIX I: SCIENTIFIC COORDINATING COMMITTEE

Mrs Saffiatou Darboe
Clinical Microbiology Laboratory
MRC Unit The Gambia
Fajara

9 August 2016

Dear Mrs Darboe,

SCC 149601.1, Molecular and phenotypic characterization of Salmonella isolates associated with disease in The Gambia

Thank you for submitting your revised proposal dated 6 August 2016 addressing the issues raised by the SCC at its meeting held on 1 August 2016.

I have reviewed the modified proposal and the changes you have made are satisfactory. I am happy to give Chair’s approval for this project and your proposal will now be forwarded to the Ethics Committee for further consideration at their next meeting on 26 August 2016.

With best wishes,

Yours sincerely,

[Signature]

Professor Umberto D’Alessandro
Chair, Scientific Coordinating Committee

Documents submitted for review:
- SCC application form, version 1.1 – 6 August 2016
- MPhil award letter – 2 December 2015

Scientific Coordinating Committee
MRC Unit The Gambia
PO Box 273 Banjul, The Gambia
West Africa

Switchboard (+220) 4495442/6 Ext 2308 Fax (+220) 4495519/4495513
E-mail: scc@mrc.gm

Internet: http://mrcportal.mrc.gm/Committees/SCC/SitePages/Home.aspx
Webpage: https://mrcportal.mrc.gm/Committees/SCC/SitePages/Home.aspx
APPENDIX II: GAMBIA GOVERNMENT/MRCG@LSHTM JOINT ETHICS APPROVAL LETTER

The Gambia Government/MRC Joint
ETHICS COMMITTEE

27 September 2016

Mrs Saffatou Darboe
Clinical Microbiology Laboratory
MRC Unit The Gambia
Fajara

Dear Mrs Darboe

SCC 1498v1.1. Molecular and phenotypic characterization of Salmonella isolates associated with disease in The Gambia

Thank you for submitting your proposal dated 6 August 2016 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 2 September 2016.

Our Committee is pleased to approve your proposed study.

With best wishes

Yours sincerely

Dr Rodrigo Cole
Deputy Chair
Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-
- SCC approval letter – 9 August 2016
- SCC application form, version 1.1 – 0 August 2016
- MPhil award letter – 2 December 2016

The Gambia Government/MRC Joint Ethics Committee:

Mr. Malamne Sonko, Chairman
Professor Ousman Nyan, Scientific Advisor
Mr. Saffatou Darboe, Secretary
Dr. Rodrigo Cole
Dr. Ahmadou Lamin Sanneh
Mrs. Sulayone Jasseh
Miss. Fatima Jagne

Prof. Umberto D’Alessandro
Dr. Ramacula Njie
Dr. Kalifa Jobang
Dr. Janine Adjani
Dr. Monocle F. Njie
Dr. Saliha Fatima Jagne

83
## APPENDIX III: LIST OF SUPPLEMENTARY TABLES AND FIGURES

Supplementary table other serovars

<table>
<thead>
<tr>
<th>Other Serovar</th>
<th>Number</th>
<th>Disease syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Bradford</td>
<td>2</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Bradenburg</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Chester</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Dublin</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Fischerstrasse</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Give</td>
<td>3</td>
<td>Gastroenteritis, Abscess</td>
</tr>
<tr>
<td>S. Glostrup</td>
<td>1</td>
<td>Abscess</td>
</tr>
<tr>
<td>S. Grumpensis</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Gueuletapee</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Hessarek</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Hull</td>
<td>4</td>
<td>Bacteraemia, Gastroenteritis</td>
</tr>
<tr>
<td>S. Landala</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Lomita</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Marseille</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Mbondaka</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Mocamedes</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Neunkirchen</td>
<td>3</td>
<td>Abscess, Bacteraemia, Gastroenteritis</td>
</tr>
<tr>
<td>S. Okerara</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Poona</td>
<td>3</td>
<td>Abscess, Gastroenteritis</td>
</tr>
<tr>
<td>S. Rubislaw</td>
<td>2</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Seattle</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Stanleyville</td>
<td>3</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Teltow</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>S. Vinohrady</td>
<td>2</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>S. Wernigerode</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
<tr>
<td>I 1,4,12,27;g,m:1,2</td>
<td>1</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>I 1,6,14,25;y:1,5</td>
<td>2</td>
<td>Bacteraemia</td>
</tr>
<tr>
<td>I 6,7:z4,z23:-</td>
<td>1</td>
<td>Gastroenteritis</td>
</tr>
</tbody>
</table>
Appendix IV Extraction Protocol

Extraction protocol:

1. Scope and Applicability

The extraction method was used to produce sufficient genomic DNA yield for Whole Genome Sequencing (WGS) of NTS. This protocol describes the procedures for pre-lysis and inactivation of bacteria harvested from solid culture media, prior to DNA extraction using a QIAampDNA Mini kit procedure.

2. Summary of Method/Principle

The protocol describes genomic DNA extraction in which the bacterial cells in the specimens were lysed to increase bacterial DNA yield and quality while removing any PCR inhibitors (i.e., salts, proteins), simultaneously dissolving the DNA in a buffer compatible with enzymes used in downstream workflow and concentrating the DNA. The method described are modified from those in QIAamp DNA Mini Kit Protocol.

3. Safety Precautions

Safety precautions were adhered to by wearing appropriate laboratory personal protective gear.

4. Equipment and Materials

Materials Required

- Pipettes (P1000, P200, P20, and P2)
- Microbiological Class II safety cabinet
- Fridges
- Freezers (-20°C, -70°C)
- Heating blocks(150°C)
- Vortex
- Microcentrifuge
- 50 ml Falcon tubes
- 1.5 ml micro-centrifuge tubes (sterile, DNase free, or PCR grade)
- Pre-sterilized Filter tips (10 μl, 200 μl, and 1000 μl)
- Sterile loops
- Single vent sterile culture plates.
5. Reagents and Chemicals

- QIAampDNA Mini Kit (Qiagen, Cat # 51306)
- Proteinase K (20mg/ml) (Qiagen, Cat # 51306)
- RNaseA Enzyme (20mg/ml) (Cat # 12091021)
- Molecular graded ethanol (96-100%)
- 20% Glycerol
- 10% bleach (10:1, distilled water: concentrated bleach)
- 70% ethanol
- DNAse free ddH2O
- Blood agar plates
- Tryptone Soya Broth (TSB) (Cat # 10525943) Thermo Scientific Oxoid

6. Preparation of TSB

This was done by dissolving 15.0 g of TSB in 500 ml ddH2O and gently mixing. This was then sterilized by autoclaving at 121°C for 15 minutes and cooling at 45°C in a water bath. The broth was then dispensed into 10ml universal bottles and quality control (QC) done before storing in a fridge 2-8°C.

7. Procedure for Extraction

7.1. A colony of NTS from an overnight MacConkey plate was inoculated into 1.5ml of TSB and incubated overnight at 37°C.

7.2. An aliquot of 1ml of the overnight liquid culture (TSB) was transferred into a 1.5ml microcentrifuge tube.

7.3. This was then centrifuged at 16,000g (12000 rpm) (14000 rpm) for 2 minutes to pellet the cells. Supernatant was removed with a pipette and carefully decanted without touching or disturbing the pellet.

7.4. Buffer ATL Solution (180 µl) was added and gently mixed by vortexing until the cells were resuspended.

7.5. Proteinase K (20 µl) was then added and mixed by gently vortexing. This was later incubated at 56°C for 1 hour on a heating block. The tubes were then centrifuge briefly to remove drops from the inside of the lid. At this stage, the heating block was set to 70°C for the subsequent steps.
7.6. Buffer AL (200 μl) was added to the sample, mixed by pulse-vortexing for 15 sec, and incubated at 70°C for 10 mins on a heating block. The tubes were briefly centrifuged to remove drops from inside the lid.

7.7. Absolute ethanol (200 μl) was later added to the samples and mixed by pulse-vortexing for 15 sec and briefly centrifuging the tubes to remove drops from inside the lid.

7.8. The contents carefully transferred to a QIAamp Mini spin column in a 2 ml collection tube. The caps closed and centrifuged at 6000 x g (8000 rpm) for 1 min. The contents were again transferred into a clean 2 ml collection tube and the filtrate discarded in the old collection tube.

7.9. Buffer AW1 (500 μl) was added to the QIAamp Mini spin column and centrifuged at 6000 x g (8000 rpm) for 1 min with the lid closed. The QIAamp Mini spin column was transferred to a clean 2 ml collection tube and the filtrate discarded in the old collection tube.

7.10. Buffer AW2 (500 μl) was then added to the QIAamp Mini spin column and centrifuged at full speed 20,000 x g: (14,000 rpm) for 3 min.

7.11 The QIAamp Mini spin column was placed in a new 2 ml collection tube and the filtrate discarded in the old collection tube. It was placed in a new tube and further centrifuged at full speed for 1 min to eliminate any trace of Buffer AW2 and the collection tube containing the filtrate discarded.

7.12 The QIAamp Mini spin column was then placed in a clean pre-labelled (lab number, sample ID, sample type) 1.5 ml Microcentrifuge tube. Buffer AE (200 μl) was added to the QIAamp Mini spin column and incubate at room temperature for 5 min before centrifuging at 6000 x g (8000 rpm) for 1 min. The spin column was then discarded.

7.16 The Qubit was used to quantify the DNA.

8. Procedure for the Qubit assay

8.1 The tube lids were pre-labelled and the Qubit Fluorometer calibrated with the required standards inserted in the order: Standard 1, followed by standard 2.

8.2 Qubit working solution. Sufficient Qubit working solution to for the standards and samples was prepared. Each standard tube requires 190 μL of Qubit working solution, and each sample tube requires anywhere from 180–199 μL make up to a final volume of 200 μL.
8.3 190 μL of Qubit working solution was added to each of the tubes used for standards before 10 μL of each Qubit standard was added to the appropriate tube. This was then mixed by vortexing 2–3 seconds while being careful not to create bubbles.

8.4 Qubit working solution was added to individual assay tubes so that the final volume in each tube after adding sample was 200 μL.

8.5 **Sample volume** between 1–20 μL was added to corresponding volumes of Qubit working solution to each assay tube between 180–199 μL to make up to 200 μL. It was then mixed by vortexing 2–3 seconds.

8.6 The tubes were allowed to incubate at room temperature for 2 minutes before “Reading standards and samples”; as per manufacturer's instructions.

8.7 **Mode of Operation**

A. On the Home screen of the Qubit 3.0 Fluorometer, press DNA, then select dsDNA High Sensitivity as the assay type. The “Read standards” screen is displayed. Press Read Standards to proceed.

   **Note:** If you have already performed a calibration for the selected assay, the instrument prompts you to choose between reading new standards and running samples using the previous calibration.

   If you want to use the previous calibration, skip to step 5.4. Otherwise, continue with step 5.2

B. **Insert** the tube containing Standard #1 into the sample chamber, close the lid, and then press Read standard. When the reading is complete (~3 seconds), remove Standard #1.

C. **Insert** the tube containing Standard #2 into the sample chamber, close the lid, and then press Read standard. When the reading is complete, remove Standard #2. The instrument displays the results on the Read standard screen. For information on interpreting the calibration results, refer to the Qubit 3.0 Fluorometer User Guide.

D. Press Run samples.

E. **On** the assay screen, select the sample volume and units: a. Press the + or – buttons on the wheel to select the sample volume added to the assay tube (from 1–20 μl). b. From the dropdown menu, select the units for the output sample concentration.

F. Insert a sample tube into the sample chamber, close the lid, and then press Read tube. When the reading is complete (~3 seconds), remove the sample tube. The instrument displays the results on the assay screen. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration.
G. Repeat step f until all samples have been read.

H. Record the DNA concentration on an excel sheet
APPENDIX V: PUBLISHED ARTICLES


Saffaatun Garbaa,1,2* Umarah Gasser,3,4 Abdi-Allah Muhmmad,3,4 Summy Cessay,5 Mamadee Jallow,5 Etta Jalo,5 J. Manu,5 Senesu Jakar,6 Idris Ayamba,2 Brenda Amoah-Adams,3 Samuel Kariie,5 Martin Antonio,7 Richard E. Bradley,9 Karen Ferreti,4 Thushan I. De Silva,1 Behzadrejesh Joseph Lorenzo1

Background. Invasive bacterial diseases cause significant disease and death in sub-Saharan Africa. Several are vaccine preventable, although the impact of new vaccines and vaccine policies on disease patterns in these communities is poorly understood owing to limited surveillance data.

Methods. We conducted a hospital-based surveillance of invasive bacterial diseases in The Gambia where blood and cerebrospinal fluid (CSF) samples of hospitalized participants were processed. Three surveillance periods were defined in relation to the introduction of pneumococcal conjugate vaccines (PCVs), before (2005–2009), during (2010–2011) and after (2012–2015) PCV introduction. We determined the prevalences of commonly isolated bacteria and compared them between the different surveillance periods.

Results. A total of 14,715 blood and 1,038 CSF samples were collected over 11 years; overall, 1,045 clinically significant organisms were isolated from 957 patients (973 organisms [6.6%] from blood and 73 [6.6%] from CSF). The most common blood culture isolates were Streptococcus pneumoniae (24.9%), Staphylococcus aureus (22.0%), Escherichia coli (10.9%), and non-typhoidal Salmonella (10.0%). Between the pre-PCV and post-PCV eras, the prevalence of S. pneumoniae bacteremia dropped across all age groups (from 32.0% to 16.5%, odds ratio, 0.43; 95% confidence interval, 0.26–0.71) while S. aureus increased in prevalence, becoming the most prevalent bacteria (from 16.0% to 27.2%, 1.75; 1.26–2.44). Overall, S. pneumoniae (53.4%), Neisseria meningitidis (13.7%), and Haemophilus influenzae (12.3%) were the predominant isolates from CSF. Antimicrobial resistance to common antibiotics was low.

Conclusions. Our findings demonstrate that surveillance data on the predominant pathogens associated with invasive disease is necessary to inform vaccine priorities and appropriate management of patients.

Keywords. Invasive bacterial disease; bacteraemia; meningitis; community-acquired infection; vaccine preventable disease.

Invasive bacterial diseases (IBDs) are a leading cause of disease and death, especially among children <5 years of age [1, 2]. Sub-Saharan Africa (SSA) carries a disproportionate burden of these diseases and associated deaths [3]. In addition, microbiological facilities and expertise are scarce and available in many SSA settings [4]. Limited epidemiological data, necessary to inform prevention strategies, show that several pathogens are associated with IBDs in SSA with diverse distribution profiles across different age groups [2, 5]. The routine use of childhood vaccines, such as Haemophilus influenzae type b (HiB) conjugate vaccine and pneumococcal conjugate vaccines (PCVs) [6, 7], has substantially modified the epidemiological profile of disease due to the steep reduction in the prevalence of these 2 bacteria in different age groups [7, 8].

In The Gambia, with the introduction of routine infant immunization with the HiB vaccine in 1997 [6], invasive HiB disease was reduced to negligible levels [10], although a small resurgence was described more than a decade later [11–13]. Subsequently, Streptococcus pneumoniae, Staphylococcus aureus, E. coli, and non-typhoidal Salmonella (NTS) became the leading causes of bacteremia in the country [14]. In August 2005, a 7-valent PCV was introduced as part of the Expanded Programme on Immunisation (EPI); it was replaced by the 13-valent PCV (PCV13) in 2011. The introduction of PCV13 reduced the incidence of invasive pneumococcal disease by 55% among young children in The Gambia [15]. The impact of PCV13 introduction in The Gambia on bacterial diseases in older children and adults, owing to the vaccine’s herd effect, has not yet been described.

The increasing threat from antimicrobial resistance remains a global challenge, resulting in longer durations of illness, mortality, and prophylactic failure [16]. This is particularly so in SSA, where the burden is substantial, the choice of effective antimicrobials is limited, and surveillance data for invasive
infections are lacking [17–19]. In addition to data on the prevalent bacterial pathogens associated with community and hospital-acquired invasive disease, hospital-based surveillance in resource-limited settings should therefore include resistance patterns to commonly used antibiotics.

This study provides data on the main causes of post-neonatal IBD in The Gambia and antibiotic susceptibility patterns over an 11-year period, between 2005 and 2015, during which PCV was introduced in the country. The data presented here are part of ongoing facility-based IBD surveillance.

METHODS AND MATERIALS

Study Setting and Population

The Gambia is a sub-tropical country in West Africa with a single wet season from June to October. Malaria is endemic, and peak transmission occurs from July to November during the rains; however, because of scaling up of malaria control interventions, a substantial decline has been observed in recent years [20]. Malnutrition remains a problem, with the prevalence of underweight, stunting, and wasting among children <5 years old estimated at 16.4%, 25.0%, and 4.3%, respectively [21]. The prevalence of human immunodeficiency virus (HIV) among adults aged 15–49 years remains low and was estimated at 2.1% in 2015 [22]. Vertical transmission is low among mothers with HIV receiving prophylaxis and treatment [23]. Infant EPI vaccine coverage is high, above 95% for the BCG vaccine and above 90% and 80%, respectively, for a single dose and 3 doses of the diphtheria-pertussis-tetanus vaccine in all regions [24].

This hospital-based surveillance was conducted at the Clinical Services Department (CSD) of the Medical Research Council (MRC) Unit The Gambia (MRCG) at the London School of Hygiene and Tropical Medicine, situated 12 km from the capital, Banjul. The CSD has provided primary- and secondary-level care to sick individuals from the surrounding population. MRCG research study participants, a small number of patients referred from other clinics since the late 1950s. Approximately 20,000 patients of all ages are seen each year in the outpatient department, and 1400 are hospitalized in the 42-bed ward. It is the only health facility in The Gambia where microbiological cultures are routinely obtained in patients with suspected IBD. Blood and cerebrospinal fluid (CSF) samples are routinely collected for bacterial culture from patients with suspected sepsis and meningitis.

Patients with suspected sepsis are treated empirically with ampicillin and gentamicin, and those with suspected meningitis are treated empirically with ceftriaxone. Treatment is subsequently modified by clinical response and laboratory results. MRCG research study participants are recruited from other surrounding health facilities, with clinical samples sent to the MRCG clinical laboratories for processing using similar methods to those used for non-research patient studies. Blood and CSF samples are collected only from referred patients who require admission on the MRC ward.

Microbiological Procedures

As part of this surveillance, bacterial isolates were obtained from blood using an automated blood-culture system (BACTEC 9050; Becton Dickinson), following the manufacturer’s instructions for quality control and blood volume requirements. Commercially produced BD BACTEC PEDS Plus F culture bottles were used for specimens obtained from children (aged 1 month to 15 years) and BD BACTEC Plus Aerobic/F and Plus Anaerobic/F culture bottles for specimens from adults (aged >15 years), as described elsewhere [14, 27]. CSF samples were processed according to World Health Organization protocol [28]. Standard microbiological procedures were performed, as described elsewhere for all pathogens [16]. In summary, pneumococcal isolates were identified using optochin disk susceptibility tests on blood agar in 5% carbon dioxide and bile solubility tests to confirm resistant isolates [27]. Haemophilus influenzae were serotyped by latex agglutination [10], and S. aureus were identified using coagulase and mannitol. For other isolates, further identification was done in the appropriate for the pathogen. All normal skin flora isolates (coagulase-negative staphylococci, Bacillus species, Micrococcus spp., diptheroids, Propionibacterium spp., and Bacillus spp. other than Bacillus anthracis) were regarded as clinically non-significant.

Antimicrobial sensitivity patterns were determined by means of Kirby-Bauer disk diffusion on Mueller-Hinton agar and interpreted according to the relevant Clinical and Laboratory Standards Institute guidelines on antimicrobial agents [32]. Antibiotics tested as relevant to each pathogen included ampicillin, gentamicin, tetracycline, cotrimoxazole, chloramphenicol, ciprofloxacin, cefotixin (to infer susceptibility to methicillin), and cefotaxime (BD Oxoid). Appropriate American Type Culture Collection controls were consistently used for the antibiotic susceptibility testing. Invasive bacteria isolates (blood and CSF) were stored at -70°C as part of routine microbiological surveillance. Samples were processed at the clinical microbiology laboratory, which is Good Clinical Laboratory Practice (2010) and ISO (International Organization for Standardization) 15189 (2015) accredited and submits to the external quality control assessment of the Kenya Accreditation Service in accordance with international quality systems for laboratories.

Statistical Analysis

Data were extracted from clinic and laboratory databases for research study participants and non-research study patients. All
relevant invasive bacterial isolates within the study period were included in the analysis. We assumed each presentation of a patient as independent but considered patients with multiple positive cultures with the same pathogen, obtained within 4 weeks of each other, as the same episode, which was therefore only reported once. We also considered an observation to be repeated if the same patient was associated with different specimen types (blood and CSF) sample.

Bacterial etiology patterns and trends were compared between surveillance periods defined by the introduction of PCVs, as follows: before PCV introduction (January 2005 to December 2009), PCV introduction and rollout (January 2010 to December 2011), and after PCV introduction (January 2012 to December 2015). Crude odds ratios for IBD, comparing post-PCV and pre-PCV periods, were obtained using logistic regression. To address confounding by age, we stratified the analyses by age. Missing values were excluded likewise, and because $<1\%$ of variables had missing values, imputation methods were not considered, and a complete case analysis was deemed adequate. The descriptive analysis for this study were carried out using Stata 13.1 software and the R Version 3.4.4 PUDIY package for the graphs.

**Ethical Review and Approval**
Clinical samples were collected for standard clinical management, and the results were anonymized for analytical purpose. The surveillance received ethical approval from the joint MRC/Gambia government ethics committee.

**RESULTS**
Between January 2005 and December 2015, a total of 14,715 blood and 11,033 CSF samples were processed for bacterial culture (Figure 1). The median age of patients with IBD was 4 years (interquartile range, 1–31 years).

**Bacteremia**
The number of blood culture samples processed was higher during the pre-PCV period (2005–2009) than during the post-PCV period, peaking in 2008 (Figure 2A). Of the samples cultured, 1876 (12.7%) were positive for any pathogen, of which 972 (51.8%) were considered clinically significant and 904 (48.2%) clinically nonsignificant or contaminants. The overall prevalence of clinically significant bacteremia was 6.6% (972 of 14,715) and ranged from 5.6% in the pre-PCV to 8.5% in the post-PCV period. The predominant clinically significant bacterial isolates in blood cultures were *S. pneumoniae* (19% [242 of 972], *S. aureus* (21% [214 of 972]), *E. coli* (11% [106 of 972]), and NTS (10% [97 of 972]) (Figure 1). We also observed a seasonal pattern of infections, with *S. pneumoniae* more common during the dry season and *S. aureus* infections more common during the wet season (Supplementary Figure 1). However, exceptions were observed in 2010 and 2011 for *S. aureus*, where the overlap started, and in 2014 for *S. pneumoniae*, which saw a steep surge in prevalence (Figure 2A). No distinct seasonal pattern was observed with other organisms.

Overall and age-specific prevalences of all blood culture isolates by vaccine surveillance period are summarized in Table 1. In addition to the significantly decreased odds of *S. pneumoniae* bacteremia (odds ratio, 0.41; 95% confidence interval, 0.29–0.58) and increased odds of *S. aureus* bacteremia (1.75; 1.26–2.44) among all age groups in the post-PCV surveillance period, we observed a 9-fold increase in the odds of bacteremia due to *N. meningitidis*. We observed no change in the *E. coli* but did note a decrease in NTS. When results were stratified by age, the most significant reduction in *S. pneumoniae* bacteremia was noted among children aged 2–23 months and adults 215 years. Likewise, the increases in *S. aureus* bacteremia was among children aged 2–23 months. No differences were observed between study periods for other common bacteria. The prevalence of the predominant pathogens by year is summarized in Figure 2A and shows a sustained increase on *S. aureus* bacteremia over the study periods along with a decline in *S. pneumoniae*.

**Meningitis**
Eighty-three (7.5%) of CSF samples cultured were positive, the majority (67.9% [73 of 108]) of which were considered clinically significant, representing 6.6% of the overall samples. Overall, the most common clinically significant isolates were *S. pneumoniae* (53% [39 of 74]), *N. meningitidis* (14% [10 of 73]), and *H. influenzae* (12% [9 of 73]) (Table 2). We observed a considerably higher number of CSF samples processed in the pre-PCV period, during which there was a corresponding increase in the isolation of *S. pneumoniae* (Figure 2B). *S. pneumoniae* was the predominant cause of meningitis in the pre-PCV period (67% [53 of 79]) compared with 29% (5 of 19) after PCV introduction; P = .002. However, the prevalence of *S. pneumoniae* meningitis gradually declined from 2008, before the introduction of PCV, *N. meningitidis* increased in the post-PCV compared with the pre-PCV period (from 61.6% to 31.6%; odds ratio, 7.08; 95% confidence interval, 1.55–32.24), becoming the most prevalent bacteria in this latter period.

**Viral Infections and Coinfections**
The same pathogen was isolated from concomitant blood and CSF samples of 36 patients (17.5, *S. pneumoniae*, 6 *N. meningitidis*, 5 *H. influenzae*, and 4 *S. aureus*). Twenty-three patients had coinfections with >1 pathogen; *S. aureus* was the most common (n = 12), coinfected with group A or group B streptococci (each n = 3), *S. pneumoniae* (n = 3), and NTS, *H. influenzae*, and *N. meningitidis* (each n = 1).

**Antimicrobial Resistance**
Antimicrobial resistance was low for the clinically relevant antimicrobial as recommended by the World Health
Organization [30], S. pneumoniae was highly sensitive to penicillin, ampicillin, and gentamicin (Figure 3A). Similarly, S. aureus was sensitive to cefoxitin, the surrogate for mecillinam and gentamicin (Figure 3B). Only 4 cases of invasive meccillinam-resistant S. aureus (1.9%) were found. In addition, resistance to gentamicin and ceftazidime was low for NTS (Figure 3C) and E. coli (Figure 3D). There was no increase in resistance over the study periods.

**DISCUSSION**

This study provides 11 years of data on the trends of the major clinically significant pathogen responsible for IBD among inpatients in urban Gambia. For the first time we have shown how these major bacteria have changed in relation to the introduction of PCV, with S. aureus and N. meningitidis replacing S. pneumoniae as the major causes of bacteraemia and meningitis, respectively. The findings of the current study underscore the need for routine hospital microbiological surveillance.

The decline of S. pneumoniae as the main cause of bacteraemia mirrors the corresponding rise in the predominance of S. aureus. Population-based surveillance in rural Gambia has shown a 75% decrease in the incidence of invasive pneumococcal disease among infants aged 2–22 months after the introduction of PCV [15]. Our hospital-based surveillance data support
the previous denominator-based findings and, in addition, show a decrease in the prevalence of invasive disease among nonvaccinated older children and adults.

Interestingly, the decrease in *S. pneumoniae* prevalence, along with the increase in *S. aureus*, started before the introduction of PCVs. The reason for these changes remain unclear, given that no major public health interventions that could have resulted in a rapid epidemiological shift were introduced around the same time. Nevertheless, years after the introduction of the wider serotype-covering PCV13, *S. pneumoniae* remains an important cause of invasive disease across all age groups (second most cause of both bacteremia and meningitis). Recent data from The Gambia have shown persistence of nasopharyngeal carriage of *pneumococci* of vaccine serotypes along with an increased carriage of nonvaccine serotypes [31]. Because carriage is a prerequisite for invasive disease, these data, along with our results, suggest that further improvement to widen the coverage of serotypes and schedule of PCV could have an additional impact.

*S. aureus* was prevalent over the surveillance period but emerged as the primary cause of bacteremia in the post-PCV era being isolated in 39% of proven bacteremia cases among infants 2–23 months old. Although our study design precludes documenting an increase incidence of *S. aureus* bacteremia, the increase in incidence of *S. aureus* bacteremia, and the global increase in incidence with a changing epidemiology attributed to a range of factors such as vaccines, virulence, invasive procedures, antibiotic resistance, and immune suppression [32, 33], underlying an urgent need for further studies and improved preventive strategies. In particular, the underlying source of *S. aureus* bacteremia in low- and middle-income countries warrants further investigation, because these may be distinct from the prevalent causes in high-income countries, such as intravenous drug use and nosocomial infections related to intravenous devices and catheters. Other factors, such as skin and soft-tissue infections, bone and joint infections, and endocarditis [34], are implicated in methicillin-susceptible *S. aureus* bacteremia.

*E. coli* and NTS were also important causes of bacteremia in our setting. Although the prevalence of *E. coli* has remained stable, that of NTS significantly declined after PCV introduction, with the overall number of cases relatively small (n = 97). This is consistent with previous reports from rural Gambia [35], in which it has been associated with the decline in malaria infection, because malaria transmission is relatively low in the study setting [20]. In addition, multiple risk factors described for invasive NTS, such as HIV infection and poor sanitation, are low in prevalence, and with documented improvement in the latter [22, 36]. Only 10 cases of meningitis due to *N. meningitidis* were observed over the entire surveillance period, and these coincided with an outbreak in the Eastern region. More than 2 decades after the Hib vaccine was introduced, with near-elimination of invasive disease in The Gambia and neighboring Senegal [10, 37], sporadic cases still occur, reinforcing the need for continued surveillance.

Antimicrobial resistance was generally low, including very few methicillin-resistant *S. aureus* isolates compared with some SSA countries [17, 38]. Antimicrobial resistance for NTS in our setting has been described elsewhere [39], and resistance to third-generation cephalosporins was low for *E. coli*. However, overall ciprofloxacin resistance for *E. coli* may warrant monitoring resistance quinolones for probable emergence of multidrug resistance [40]. Although resistance did not significantly increase over the study period, overprescription of antibiotics needs to be monitored, and stringent control measures should be in place to encourage the use of guidelines [41], because alternative antibiotics strategies are limited in resource-limited countries such as The Gambia.

The current study has several limitations intrinsic to the surveillance design. Because our data are hospital based, the
<table>
<thead>
<tr>
<th>Pathogens by Age Group</th>
<th>Total</th>
<th>PCV Period</th>
<th>PCV Introduction Period</th>
<th>PCV+Hib Period</th>
<th>Percent Change in PCV+Hib Period</th>
<th>Percent Change in PCV Period</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ages</td>
<td>972</td>
<td>494</td>
<td>126</td>
<td>252</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>262</td>
<td>102</td>
<td>22 (22.4)</td>
<td>21 (16.1)</td>
<td>0.64</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>214</td>
<td>72</td>
<td>14 (16.4)</td>
<td>21 (22.2)</td>
<td>1.76</td>
<td>1.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>105</td>
<td>35</td>
<td>11 (11.1)</td>
<td>23 (11.1)</td>
<td>0.39</td>
<td>0.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>37</td>
<td>13</td>
<td>2 (2.5)</td>
<td>5 (5.3)</td>
<td>0.65</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Other gram-negative bacteria</td>
<td>37</td>
<td>13</td>
<td>2 (2.5)</td>
<td>5 (5.3)</td>
<td>0.65</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Other gram-positive bacteria</td>
<td>37</td>
<td>13</td>
<td>2 (2.5)</td>
<td>5 (5.3)</td>
<td>0.65</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>26</td>
<td>9</td>
<td>1 (2.5)</td>
<td>5 (20.0)</td>
<td>0.72</td>
<td>0.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>25</td>
<td>8</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Haemophilus influenzae non-type b</td>
<td>9</td>
<td>3</td>
<td>1 (2.0)</td>
<td>2 (7.0)</td>
<td>0.66</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GAS</td>
<td>25</td>
<td>8</td>
<td>2 (2.0)</td>
<td>5 (20.0)</td>
<td>0.72</td>
<td>0.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. aureus</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NTS</td>
<td>21</td>
<td>7</td>
<td>2 (2.0)</td>
<td>5 (20.0)</td>
<td>0.72</td>
<td>0.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>20</td>
<td>6</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GBS</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NTS</td>
<td>18</td>
<td>6</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E. coli</td>
<td>18</td>
<td>6</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>9</td>
<td>3</td>
<td>1 (1.0)</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GBS</td>
<td>45</td>
<td>15</td>
<td>5 (11.1)</td>
<td>23 (52.2)</td>
<td>0.72</td>
<td>0.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>25</td>
<td>8</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NTS</td>
<td>11</td>
<td>3</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>9</td>
<td>3</td>
<td>1 (1.0)</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GBS</td>
<td>25</td>
<td>8</td>
<td>2 (2.0)</td>
<td>5 (3.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>14</td>
<td>4</td>
<td>1 (2.0)</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GBS</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>12</td>
<td>4</td>
<td>1 (2.0)</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>15</td>
<td>5</td>
<td>1 (2.0)</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GBS</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0.76</td>
<td>0.77</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

95
Table 1. Continued

<table>
<thead>
<tr>
<th>Pathogen by Age Group</th>
<th>Total</th>
<th>Pre-PCV Period</th>
<th>PCV Introduction</th>
<th>Post-PCV Period</th>
<th>OR (95% CI)</th>
<th>Change in Post-PCV vs Pre-PCV Periods, %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas spp.</td>
<td>4</td>
<td>2 (4.4)</td>
<td>1 (2.2)</td>
<td>1 (1.7)</td>
<td>0.97</td>
<td>0.05-3.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4 (8.8)</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>NA</td>
</tr>
<tr>
<td>H. influenzae non-type b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (8.1)</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>NA</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>1</td>
<td>1 (2.2)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>NA</td>
</tr>
<tr>
<td>GAS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CSE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NTS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Other gram-negative bacteria</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Other gram-positive bacteria</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>GAS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H. influenzae non-type B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>NA</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CSE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; GAS, group A streptococci; CSE, group B streptococci; NA, not available; NTS, nontypable Salmonella; OR, odds ratio; PCV, pneumococcal conjugate vaccine.

Table 2. Distribution of Cerebrospinal Fluid Pathogens in the Different Vaccine Periods (All Age Groups)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Total</th>
<th>Pre-PCV Period</th>
<th>PCV Introduction</th>
<th>Post-PCV Period</th>
<th>OR (95% CI)</th>
<th>Change in Post-PCV vs Pre-PCV Periods, %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>156</td>
<td>32 (0.8)</td>
<td>1 (0.2)</td>
<td>6 (1.2)</td>
<td>0.17</td>
<td>0.06-1.07</td>
<td>0.67</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>10</td>
<td>2 (1.8)</td>
<td>1 (3.5)</td>
<td>7 (8.0)</td>
<td>0.78</td>
<td>0.50-1.32</td>
<td>0.60</td>
</tr>
<tr>
<td>Hemophilus influenzae non-type b</td>
<td>0</td>
<td>1 (0.9)</td>
<td>1 (0.9)</td>
<td>1 (0.8)</td>
<td>0.80</td>
<td>0.20-3.00</td>
<td>0.63</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4 (8.1)</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>1 (1.0)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.69</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2</td>
<td>2 (1.0)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.69</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CSE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>GAS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H. influenzae non-type B</td>
<td>1</td>
<td>1 (1.0)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.68</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td>2</td>
<td>2 (1.0)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.69</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CSE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not available; OR, odds ratio; PCV, pneumococcal conjugate vaccine.

Observed changes in pathogen prevalence were dependent on sampling and may not reflect changes in incidence. It is a retrospective analysis, and any changes in cases ascertained, amount of sample volume collected, or health-seeking behavior in the population may have modified the overall yield of bacteria. Although the case ascertainment for patients seen at the MRCC GSD did not change over the surveillance period, there were several research studies during the period, each with different age inclusion criteria. Although this might not have changed the distribution of pathogens, it probably increased the number of isolates in some years compared with others. The linking of clinical data associated with microbiological findings is another limitation. In addition, we could not determine what proportion of patients had received the PCV. Finally, we determined antimicrobial sensitivity using disk diffusion without final confirmation by Etest, which may have resulted in variability.
in an overestimation of the prevalence of antibiotic resistance levels. Still, results show a low prevalence of antibiotic resistance and no trends for an increase.

Our long surveillance data have shown that S. aureus and N. meningitidis emerged as the leading causes of bacteraemia and meningitis, respectively, in urban Gambia, while S. pneumoniae remains a leading cause of IBD, even after the introduction of PCV13. The changing epidemiology of IBD makes a compelling case for regular microbiological and antimicrobial surveillance data, which is lacking in SSA. Not only are such data necessary for healthcare workers to inform appropriate antibiotic prescribing practices, but they are also vital for prioritizing vaccine development, for emerging pathogens such as S. aureus and optimizing schedules of current vaccines. New vaccines targeting S. aureus should focus on young infants and older children, among whom the prevalence of IBD is highest.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consenting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors. As questions or comments should be addressed to the corresponding author.

Notes
Author contributions. S. D., U. O., R. S. R., O. S., and A. R. designed the study and wrote the manuscript. S. D. and U. O. prepared the tables and figures. S. D. and A. K. M. analyzed the data. S. D., R. C., M. J., and O. S. performed the laboratory analysis. All authors reviewed and contributed to the manuscript. A. S. R. contributed to the manuscript in his personal capacity and in his role as an adjunct academic at Central Queensland University.

Acknowledgments. The authors thank Anh Thiem for her comments and suggestions, Katherine Lamah and Eunice Muvungi for helping retrieve records, and the staff of the Clinical Services Department and Clinical Laboratories.

Disclosure: The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the World Health Organization, the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine or the authors’ affiliated institutions.

Financial support. This work was supported by the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine.

Supplement sponsorship. This supplement was supported with funds from Gavi, the Vaccine Alliance through the World Health Organization and the GAVI Foundation, and The Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine.

Potential conflict of interest. All authors. No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential
References


Invasive bacterial infections in Gambians with sickle cell anemia in an era of widespread pneumococcal and hemophilus influenzae type b vaccination

Gormandor Soothill, BSc, MBBS, MRCP, Sallifou Darboe, BSc, Gborm Bah, MSc, Lawal Bolarinde, MSc, Aubrey Cunningham, PhD, FRCPCH, Suzy T. Anderson, MBBS, PhD

Abstract
There is relatively little data on the etiology of bacterial infections in patients with sickle cell anemia (SCA) in West Africa, and no data from countries that have implemented conjugate vaccines against both Streptococcus pneumoniae and Haemophilus influenzae type b (HiB). We conducted a retrospective analysis of SCA patients admitted to the Medical Research Council Unit, The Gambia, during a 5-year period when there was high coverage of HiB and Pneumococcal conjugate vaccines. We evaluated 161 admissions of 126 patients between April 2010 and April 2015. Pathogenic bacteria were identified in blood cultures from 11 of the 131 admissions that had cultures taken (8.4%, 95% CI 4.5%–14.1%). The most frequent isolate was Salmonella Typhimurium (6/11; 54.5%), followed by Staphylococcus aureus (2/11; 18.2%) and the other Enteric Gram-negative pathogens (2/11; 18.2%) and there was 1 case of H. influenzae non-type b bacteria (1/11; 9.1%). There were no episodes of bacteremia caused by S. pneumoniae or HiB. The low prevalence of S. pneumoniae and HiB and the predominance of non-type b Salmonella as a cause of bacteraemia suggest the need to reconsider optimal antimicrobial prophylaxis and the empirical treatment regimens for patients with SCA.

Abbreviations: CSF = cerebrospinal fluid; HiB = Haemophilus influenzae type b; MR08 = Medical Research Council Gambia; NTS = nontyphoidal Salmonella; PCV = pneumococcal conjugate vaccine; SCA = sickle cell anemia.

Keywords: antimicrobial prophylaxis, bacteraemia, sickle cell anemia, West Africa.

1. Introduction
Patients with sickle cell anemia (SCA) are at increased risk of bacterial infections, which are leading causes of morbidity and mortality, especially in populations that lack effective prophylaxis and treatment. Although >90% of people with SCA are

born in Africa, paradoxically there is relatively little high quality data on infectious complications in these populations. Historically, studies from resource-rich settings have identified Streptococcus pneumoniae, Haemophilus influenzae type b (HiB), and nontyphoidal Salmonella (NTS) species to be the most common invasive bacteria.[1–5] Studies from Africa have produced inconsistent results, with a large study from Kenya finding similar pathogens to those seen in western populations,[6] whereas studies from Uganda, Nigeria, and more recently Tanzania, showing that Staphylococcus aureus, NTS species, and other Enteric Gram-negative pathogens predominated.[7–9] There are limited data about types of pathogens and efficacy of antimicrobial prophylaxis in West Africa and no published data from SCA patients in The Gambia. Penicillin prophylaxis and conjugate vaccines against S pneumoniae and HiB have significantly improved prognosis and almost eliminated the additional risk of bacteraemia associated with SCA in resource-rich settings.[4, 10, 11] However, these are not routinely implemented in much of West Africa, despite the greatest global burden of disease.[12] The Gambia is relatively unique in that HiB and pneumococcal conjugate vaccines are well established as part of the Expanded Program on Immunization.[13] The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2009, followed by the 13-valent vaccine (PCV13) in 2013,[14] and the estimated proportion of Gambian infants receiving all 3 pentavalent vaccines (containing HiB) and PCV 13 is 89.3%.[15]

Based on the pathogen distribution found in recent studies, some commentators have challenged the benefits of penicillin
prophylaxis in African populations. We aimed to review the predominant pathogens causing invasive bacterial infections in SCA patients admitted to the Medical Research Council Gambia (MRCG) Unit clinical wards through retrospective analysis of patient and laboratory records.

2. Methods

2.1. Setting and population

This study was conducted at the Clinical Services Department (CSD) of the MRCG, located in the urban, western coastal region where malaria transmission is relatively low. The Gambia is a small country (population 1.8 million in 2015) situated north of the equator in West Africa. National prophylaxis guidance for SCA recommends pyrimethamine (for malaria) and phenoxymethylpenicillin.

The CSD at the MRCG comprises an outpatient medical clinic seeing around 50,000 patients a year and a 42-bed inpatient unit. All patients with history or clinical findings suggestive of SCA are screened using a microscope test, followed (if positive) by hemoglobin electrophoresis. There is no specific protocol for management of SCA complications; however, blood cultures are drawn from all patients with clinical features compatible with invasive bacterial infection. Other samples from sterile sites (e.g., cerebrospinal fluid [CSF]) are collected when clinically indicated. A high-quality, clinical diagnostic laboratory is located at the CSD and provides round-the-clock microbiology, immunology, and virology services. Empirical antibiotic treatment for suspected septis is ampicillin and gentamycin.

2.2. Ethics

Ethical review was not required for this study since it was undertaken as a clinical audit to establish the prevalence and causes of invasive bacterial infections in SCA patients at the MRCG CSD.

2.3. Data collection

The admission records of all patients presenting to the CSD over a 5-year period (17th April 2010 to 27th April 2015) were reviewed. Patients with SCA were identified from discharge diagnosis records. We recorded whether patients were known to have SCA, what medications were taken prior to admission, hemoglobin values, temperature, duration of admission, clinical diagnosis, bacteremia or other positive sterile site culture, and sensitivities of cultured pathogens.

We defined the season as June to November and dry season as December to May. Severe anemia was defined as hemoglobin <50 g/L. For cultural and practical reasons, all patients admitted had axillary temperature measured, and for the purpose of this study, a fever cutoff value of ≥37.5°C was selected, which has been shown to increase sensitivity for predicting infection without greatly impacting specificity.

2.4. Laboratory methods

SCA was diagnosed using hemoglobin electrophoresis. Full blood count analysis for each patient’s sample was performed using either the automated Medonic M Series, 5 part differential Hematology Analyzer or the automated Celldyn 3700, and 5 part differential Hematology Analyzer.

Blood cultures were performed using the BACTEC 9600 automated culture system. In total, 1 to 3 mL blood was inoculated into 20 BACTEC Peds PLUSF culture for children and 3–10 mL in each aerobic and anaerobic vial for adults. Bottles were usually placed in the incubator within 2 hours of collection.

If a delay occurred, bottles were pre-mixed at 35–37°C. Microbiological procedures were performed using standard media formats and biochemical tests, and serological agglutination. Staphylococcus isolates were identified by coagulase, mannitol fermentation, and Catalase tests. Salmonella Typhimurium was identified using the bioMerieux analytical profile index: API 20 E (Becton Dickinson, Sparks, MD), and characterized by serotyping, using Shee strain reference Salmonella Sera-QQuick kit (groups A-G) and Salmonella Sera-QQuick ID kit (specific for Typhimurium and Enteritidis). Other enterics Gram-negatives were identified using the bioMerieux analytical profile index: API 20 E. Antibiotic susceptibility was assessed according to CLSI interpretation guide. For the purpose of this study, all organisms found as normal skin or oral flora were considered to be contaminants, including coagulase-negative Staphylococci, alpha-hemolytic Streptococci (other than S pneumoniae), and diphtheroids.

2.5. Statistical methods

Each hospitalization was considered as a separate event, although some patients were admitted more than once. Results are reported as proportions of total number of admissions, blood cultures or isolates, or as median values and interquartile ranges for quantitative variables. Where appropriate, the “known” package in R was used to calculate Jeffrey’s 95% confidence intervals for proportions. The Mann–Whitney U test was used to compare the hematological parameters of patients with and without a proven invasive bacterial infection. P value of <0.05 was used to define a significant difference.

3. Results

Data from 116 admission episodes of 126 patients with SCA were included in the analysis (Fig. 1). A summary of characteristics is shown in Table 1. Of 23 patients with >1 admission, the median time between discharge and readmission was 87 days and
### Table 1

Patients' characteristics by the presence of invasive bacterial infection.

<table>
<thead>
<tr>
<th></th>
<th>All subjects n=161 (number, %)</th>
<th>No proven invasive bacterial infection n=146 (number, %)</th>
<th>Proven invasive bacterial infection n=15 (number, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 y</td>
<td>5 (3.1)</td>
<td>5 (3.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1–2 y</td>
<td>25 (15.5)</td>
<td>17 (11.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>3–6 y</td>
<td>41 (25.3)</td>
<td>30 (20.7)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>7–15 y</td>
<td>50 (30.2)</td>
<td>57 (39.4)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>&gt;15 y</td>
<td>37 (23.0)</td>
<td>36 (24.7)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86 (53.4)</td>
<td>77 (52.7)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Female</td>
<td>75 (46.6)</td>
<td>69 (47.2)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>76 (46.7)</td>
<td>71 (48.6)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Dry</td>
<td>80 (49.3)</td>
<td>75 (51.4)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New diagnosis</td>
<td>76 (47.2)</td>
<td>67 (45.9)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Known SCA</td>
<td>85 (52.8)</td>
<td>73 (44.1)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td><strong>Medications on admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>165 (95.2)</td>
<td>95 (65.1)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td>Rifaximin only</td>
<td>16 (9.9)</td>
<td>10 (6.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Piperacillin only</td>
<td>8 (4.9)</td>
<td>6 (4.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Piperacillin + Phenoxymyzamin only</td>
<td>1 (0.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Rifaximin + Piperacillin</td>
<td>32 (19.9)</td>
<td>28 (19.2)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Rifaximin + Piperacillin + Phenoxymyzamin</td>
<td>1 (0.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Rifaximin + Phenoxymyzamin + Piperacillin</td>
<td>2 (1.2)</td>
<td>2 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (2.5)</td>
<td>3 (2.1)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td><strong>Length of admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 d</td>
<td>67 (41.6)</td>
<td>67 (45.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>3–5 d</td>
<td>65 (40.4)</td>
<td>62 (42.5)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>≥10 d</td>
<td>26 (16.1)</td>
<td>16 (11.0)</td>
<td>12 (80.0)</td>
</tr>
<tr>
<td>Died</td>
<td>1 (0.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>164 (99.7)</td>
<td>13 (9.0)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>&lt;10 g/l</td>
<td>36 (22.1)</td>
<td>26 (17.9)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>≥10 g/l</td>
<td>121 (74.2)</td>
<td>100 (70.4)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td><strong>Temp on admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>122 (76.0)</td>
<td>10 (7.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>&lt;37.5 °C</td>
<td>91 (55.8)</td>
<td>80 (54.9)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>37.5–38.5 °C</td>
<td>76 (47.3)</td>
<td>71 (48.6)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>≥38.5 °C</td>
<td>26 (16.1)</td>
<td>20 (13.5)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td><strong>Specific clinical diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaso-occlusive crisis</td>
<td>62 (38.0)</td>
<td>53 (36.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>15 (9.3)</td>
<td>10 (6.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>17 (10.5)</td>
<td>6 (4.1)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Septic unknown source</td>
<td>13 (8.0)</td>
<td>10 (6.9)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Vaso-occlusive crisis + septic unknown source</td>
<td>9 (5.5)</td>
<td>6 (4.1)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>8 (5.0)</td>
<td>7 (4.8)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Septic shock / sepsis</td>
<td>1 (0.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Septic shock / sepsis + malaria</td>
<td>7 (4.3)</td>
<td>4 (2.7)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>Anemia</td>
<td>6 (3.7)</td>
<td>6 (4.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vaso-occlusive crisis + malaria</td>
<td>5 (3.1)</td>
<td>5 (3.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Malaise</td>
<td>4 (2.5)</td>
<td>4 (2.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>4 (2.5)</td>
<td>4 (2.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>2 (1.3)</td>
<td>2 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Acute heart syndrome</td>
<td>2 (1.3)</td>
<td>2 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Acute respiratory illness</td>
<td>2 (1.3)</td>
<td>2 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (6.8)</td>
<td>10 (6.9)</td>
<td>1 (6.7)</td>
</tr>
</tbody>
</table>

SCA = septic cell anemia.
1. Metastatic and solid lesions, post-infective arthritis, acute pancreatitis, septic shock and sepsis, cardiac vascular accident, gastroenteritis, myositis, meningitis, nephritis, respiratory syndromes, nervous system.

101
the minimum interval was 1 day. Five patients were readmitted within 1 week of discharge; no culture positive patient were recounted for the same bacterial infection. One patient had 2 admissions with positive blood cultures 1 month apart, S aureus was cultured on their first admission and H influenzae type B on their second; they had a negative HIV test. The median length of admission was 5 days, and there was 1 death of a patient diagnosed with an aplastic crisis with negative blood cultures. The median age of patients was 5 years (interquartile range [IQR]: 2–13 years). Just under half of patients had a new diagnosis of SCA made on admission (median age 4 years [IQR: 2–6 years]). There was no statistically significant difference in the laboratory parameters of patients with and without a proven invasive bacterial infection (Table 2).

Of the 161 admission episodes, 131 (81%) had blood cultures taken, and 14 (12.2%, 95% CI 7.4–18.6%) of these were positive. Five blood cultures (3.8%, 95% CI 1.5–8.2%) grew suspected contaminants and 11 (8.4%, 95% CI 4.5–14.1%) yielded pathogenic organisms. The most frequent pathogenic isolates were NTS (67; 54.5%), other enteric Gram-negative (22; 11.8%), and S aureus (21; 11.8%). No episodes of S pneumoniae or Hib bacteremia were identified (91.1%, 95% CI 0.1–1.5%), but there was 1 case of H influenzae non-type b (7.1%) of positive blood cultures occurred in children aged under 5. Cultures from other sterile sites (CSF and pus aspirates) were also analysed, revealing 4 additional positive cultures (Fig. 2). One child with a new diagnosis of SCA, unknown immunization status, and not taking penicillin prophylaxis, had S pneumoniae isolated from CSF.

4. Discussion

SCA is estimated to be one of the biggest causes of premature death in West Africa, mainly attributed to infections. The prevalence of SCA in The Gambia is unknown, and the lack of prevalence data and the failure of children to be routinely screened either at birth or on hospital admission means that it is likely to be significantly underestimated. Four small cross-sectional surveys completed in rural Gambia estimated the percentage of newborns with SCA to be between 0.8% and 1.2%, with a high excess mortality in early childhood.

Knowledge of the most common pathogens infecting patients with SCA could be used to improve antimicrobial prophylaxis and empirical treatment of infections. Variations in the prevalence of S pneumoniae and Hib in studies of bacteremia in SCA patients in Africa have been the cause of much debate.

In this study, we found no cases of S pneumoniae or Hib bacteremia. One case of S pneumoniae was identified from CSF culture, and there was 1 case of H influenzae non-type b bacteremia. S pneumoniae and Hib infections are more common among younger SCA patients, and it has been suggested that studies of known SCA patients in sub-Saharan Africa are missing infections occurring in younger children due to late diagnosis of SCA. Although there are regional screening programs for SCA in sub-Saharan Africa, their coverage is minimal and diagnosis is usually made when a complication occurs. The median age in our study was 5 years, whereas that of children diagnosed on presentation (just under half of subjects) was 4 years, so it is possible that the low rates of S pneumoniae and Hib in this study are a reflection of this older age range and survivorship bias.

The Gambia has a well-established vaccination program for SCA patients, and the use of Hib conjugate vaccine is known to reduce the incidence of bacteremia in children. Therefore, it is highly likely that the prevalence of S pneumoniae and Hib infections is lower than other countries. A study conducted at MRC Gambia before introduction of the PCV (2003–2005) found that S pneumoniae accounted for 45.2% of community acquired bacteremia in children, followed by...
S. pneumoniae (18.3%), E. coli (9.7%), and NTS (8.6%) [2]. In contrast, a review of cases of bacteremia in the same unit after the establishment of these vaccination programs (2010–2014) showed a decline in S. pneumoniae to just 15.1%, with S. aureus and the predominant pathogen accounting for 24% of isolates (S. Anderson, unpublished data). This trend is mirrored by recent data from elsewhere in The Gambia. [23]

It is unlikely that the low yield of S. pneumoniae observed in this cohort of patients is due to inadequate culture facilities. In contrast to many other low-resource settings, the MRG has excellent clinical management and basic microbiology laboratory. However, because this was a retrospective study it was not possible to determine time from blood collection to inoculation, or the volume of blood collected for culture—both factors impact on culture yield and likelihood of recovery. Nonetheless, the rate of blood culture contamination (3.8% of blood cultures taken and 31% of positive blood cultures) is lower than other studies in sub-Saharan Africa, [24] and suggests poor technique is unlikely to have greatly influenced our results.

The retrospective nature of the study means we did not have some information that could be important for interpreting our results (immunization status; clinical justification for taking blood cultures; the decision making process used to assign a final diagnosis; or the phenotype of the pneumococcal meningitis isolate). We also do not have details of pre-vaccination data. We do not know for certain about prior antimicrobial treatment, which may have resulted in negative culture results. However, only 5% of patients were documented as taking pneumococcal penicillin prophylaxis on admission, suggesting this effect may be small. Rates of bacteremia may also have been underestimated because blood cultures were not performed on all patients. However, our results are consistent with a recent study in Tanzania, where similar rates of bacteremia (4.8% of admissions) and distribution of pathogens (S. aureus 28%, NTS 21%, S. pneumoniae 17%) were identified. [7] Records were unavailable for nearly one-third of potentially eligible cases, and we do not know what effect this might have had on our results.

Despite the limitations of this study, it is remarkable that there were no cases of S. pneumoniae or Hib bacteremia. The predominance of NTS species and other enteric Gram-negatives suggest a need to reassess previous antimicrobial prophylaxis and empirical treatment in this population. [25] Despite its success in resource-rich settings, penicillin prophylaxis may not be optimal for PCV and Hib-vaccinated patients with SCA in The Gambia. However, it is unclear whether possible alternative regimens such as cotrimoxazole or azithromycin would be safe or cost-effective. For SCA patients with suspected sepsis, empirical treatment must be effective against both NTS and S. aureus, and account for local resistance patterns. At other countries in sub-Saharan Africa adopt PCV, they may see a changing spectrum of pathogens in SCA patients. We suggest that further research, including clinical trials, is needed to determine locally appropriate treatment and prophylaxis regimens for SCA.

Acknowledgment

The authors are grateful to the staff of MRG GSD laboratories, and clinical registry.

References


Brenda Kwambane-Adams,1,4 Saffieta Darboe,1,4 Helen Nalwera,1,5 Ebenezer Festor Nyarko,3 Usman N. Kamaro,4 Osunna Sackey,1 Mudjeepa Betts,6 Richard Bradbury,7 Rita Wnekuller,3 Boldane Luval,6 Dabashah Saha,1 M. Jahaqir Hussain,4 Andrew M. Prontici,1 Beat Koemmo,3 Suzanne Anderssen,7 Umberto Dustinian,4,5 and Martin Selby1,4,9

1Medical Research Council Unit, Fajara, and International Nutrition Group, Medical Research Council Unit, Banjul, The Gambia, 2School of Medical and Applied Sciences, Central Queensland University, North Rockhampton, Australia, 3Institute of Tropical Medicine, Antwerp, Belgium, 4Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, and 5Microbiology and Infectious Unit, University of Nottingham, United Kingdom

Background. There are large data gaps in the epidemiology of diseases caused by Salmonella enterica in West Africa. Regional surveillance of Salmonella infections is necessary, especially with the emergence and spread of multidrug-resistant clones.

Methods. Data on Salmonella isolated from various clinical specimens from patients from across The Gambia were collected and analyzed retrospectively from 2005 to April 2015. Antibiotic sensitivity testing of Salmonella isolates was performed by disk diffusion method. Serotyping and serogrouping of Salmonella isolates was performed using standard microbiology techniques.

Results. Two hundred thirty seven Salmonella isolates were isolated from 190 patients: 52% (106/203) from blood and 39% (79/203) from stool specimens. Salmonella was also isolated from urine, aspirates, cerebrospinal fluid, wounds, and abscesses. The prevalence of Salmonella in blood cultures was 0.8% (106/13 905). Of the serotyped salmonelles, 14% (1/15) were Salmonella enterica serovar Typhi, whereas 80% (13/15) were serovars other than Typhi (nontyphoidal Salmonella). Of the 102 typed NTS isolates, 40% (41) were Salmonella enterica serovar Typhimurium, 10% (10) were Salmonella enterica serovar Infantis, and 5% (5) were Salmonella enterica serovar Arizonae. Overall, 70% (143/203) of the salmonelles were nontyphoidal. Multidrug resistance was found in 4% (9/203) of the isolates, 3 of which were Salmonella Enteritidis.

Conclusions. Salmonella is associated with a wide spectrum of invasive and noninvasive infections across all ages in The Gambia. There is evidence of multidrug resistance in salmonellae that warrants vigilant monitoring and surveillance.

Keywords: Salmonella enterica; Typhi; nontyphoidal Salmonella enterica; multidrug resistance; invasive Salmonella disease.

Despite having among the largest burdens of Salmonella infections worldwide, there is limited comprehensive data on the prevalence and characteristics of invasive and noninvasive Salmonella disease across sub-Saharan Africa [1,2]. These data gaps are even more apparent in West Africa where epidemiological and antibiotic resistance data are scarce [1,3]. Salmonella enterica serovar Typhi causes typhoid fever and is responsible for >21 million infections and nearly 200 000 deaths annually [4]. Salmonella enterica is estimated to cause >85 million infections every year [5]. Nontyphoidal Salmonella enterica (NTS) is associated with life-threatening infections in sub-Saharan Africa including septicemia and meningitis. These serious infections appear to occur more frequently among young patients with malaria, anemia, malnutrition, and immunosuppression [6]. In recent years, the emergence and spread of multidrug-resistant (MDR) Salmonella in sub-Saharan Africa, including West Africa, has become a major public health issue [3,7].

The importance of salmonellae as a pathogen causing serious infections and the emergence of MDR strains warrant structured disease monitoring and surveillance.
across the subcontinent. Implementation of such surveillance and monitoring systems may ironically be hampered by the lack of supporting data. In The Gambia, *Salmonella* has been previously described as an important cause of serious bacterial infections [8-11]; however, these studies primarily investigated bloodstream infections (BSIs). This study provides data on the spectrum of diseases caused by *Salmonella* in The Gambia and their phenotypic and antibiotic susceptibility characteristics. The data presented here provide 10-year baseline data as a basis for structured surveillance and monitoring of *Salmonella* infections and possibly vaccine trials in The Gambia and the West Africa subregion.

**MATERIALS AND METHODS**

**Study Population and Samples**

The study was conducted in four regions in The Gambia: the Western Region, the Lower River Region, and the Upper River region (Figure 1). From 2005 to 2013, *Salmonella* were isolated from various clinical specimens including blood, stool, urine, aspirates, cerebrospinal fluid (CSF), wounds, ear swabs, and abscesses. Isolates of *Salmonella* from blood, CSF, and aspirates were defined as invasive NTS. These isolates were cultured from specimens from outpatients and inpatients of all age groups who attended the Medical Research Council (MRC) Clinic in Fajara, Western Region. Isolates from different clinical specimens (e.g., blood, CSF, and stool) from the same patients and/or clinical specimens collected at different times from the same patients were identified using the MRC Fajara clinic unique patient identifier. Each of the isolates from the same patient was assigned an laboratory identifier and processed independently. These facilities and the procedures have been described previously [9]. From 2007 to 2012, *Salmonella* were isolated from stool samples collected from children <5 years old with moderate to severe diarrhea who participated in the Global Enteric Multicenter Study (GEMS) conducted in the Upper River Region [12]. *Salmonella* was cultured from stool samples collected from malnourished children 9-24 months old from the Lower River Region of The Gambia. Information on the isolates is summarized in Table 1.

**Ethical Approval**

Ethical approval was obtained from the Joint MRCGambian Government Ethics Committee and the Institutional Review Board of the University of Maryland at Baltimore.

**Bacteriologic Methods**

Blood, CSF, and aspirate specimens were processed using a BACTEC 9600 automated blood culture system (Becton Dickinson, Toms River, New Jersey) according to the manufacturer’s instructions. In brief, 1-3 ml of specimen was inoculated into commercially produced BD BACTEC PEDS PLUS/F culture for children and 3-10 ml was inoculated in each aerobic and anaerobic vial for adults as described previously [13]. Standard microbiological procedures were performed as previously described using standard media. Other specimens including stool, urine, and wound swabs were directly cultured on xylose lysine deoxycholate agar, MacConkey agar, and selenite F broth overnight [13]. The Selenite broth was further cultured on xylose lysine deoxycholate agar for the selective culture of *Salmonella*. Any suspected *Salmonella* colony was inoculated on xylose and triple sugar iron agar slope and, if suspected to be *Salmonella*, was then tested using the API 20E bacterial identification system (bioMérieux). Serology was

---

**Figure 1.** Map of The Gambia indicating regions where *Salmonella* were isolated. The area of each circle corresponds to the number of isolates. Abbreviation: GMR Arms, Gambia Administration.
Table 1. Characteristics of Patients From Whom *Salmonella* Was Isolated Between 2010 and 2015 in The Gambia, West Africa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Category</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>West Coast Region</td>
<td>157</td>
<td>82.6</td>
</tr>
<tr>
<td></td>
<td>Upper River Region</td>
<td>30</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>Lower River Region</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>98</td>
<td>51.6</td>
</tr>
<tr>
<td></td>
<td>Stool</td>
<td>79</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>7</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Abscess/Wound</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Arthrite</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Ear swab</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;1 y</td>
<td>25</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>1 to &lt;5 y</td>
<td>64</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>5 to &lt;10 y</td>
<td>23</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>10 to &lt;15 y</td>
<td>33</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>16 to &lt;20 y</td>
<td>30</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>≥20 y</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>108</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>86</td>
<td>45.3</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Hospitalized</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>67</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>86</td>
<td>46.3</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>37</td>
<td>19.5</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>111</td>
<td>58.4</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>79</td>
<td>41.6</td>
</tr>
</tbody>
</table>

Note: CSF: cerebrospinal fluid; NA, unknown.

*Ten patients had >1 isolate collected from different sources and/or the same source at different times. Where multiple isolates were collected from the same patient, only the first isolate is included in this summary. Where multiple isolates were collected from different sites, only the most invasive is included. CSF, blood, or ascites.*

*All isolates were stored at 20°C.*

Stored isolates were retrieved and recultured on Mueller-Hinton agar. Susceptibility testing and serotyping were repeated for uniformity, as different susceptibility interpretations and antisera may have been used in the past to type the isolates. Later serotyping was done using Steven's Serum Institute *Salmonella* Sero-Quick Group kit (groups A–G) and *Salmonella* Sero-Quick ID kit for serovars Typhimurium and Enteritidis (Steven's Serum Institute, Copenhagen, Denmark) when API 20E confirmed *Salmonella* species following the manufacturer's protocol. Serotyping and susceptibility testing of the isolates were performed at the MCR Unit, The Gambia.

Antimicrobial susceptibility testing was performed by disk diffusion method targeting 7 antibiotics [16]. The pattern of susceptibility testing were determined by disk diffusion on Mueller-Hinton agar and interpreted according to Clinical and Laboratory Standards Institute guidelines on antimicrobial agents [14]. Antibiotics tested were ampicillin, gentamicin, tetracycline, cefotaxime, chloramphenicol, cephalaxin, and cefotaxime (BD Oxoid, Basingstoke, United Kingdom). *Salmonella Enteritidis American Type Culture Collection (ATCC) 13076 at Escherichia coli ATCC 25922 were used as controls for the antibiotic susceptibility testing.

**Data Management**

Patient information, provisional diagnosis, and *Salmonella* culture data were collected from microbiology records for samples from the Western Region. The data on total number of blood culture performed per year was collected from clinical microbiology logbooks. Data from GBMS [12] were collected and stored as previously described. The descriptive analyses for this study were performed using Stata version 12 and Microsoft Office Excel software.

**RESULTS**

Analysis was carried out on a total of 203 *Salmonella* isolated from 190 patients between January 2005 and April 2015 and archived at the MRC Unit, The Gambia. The characteristics of the patients are summarized in Table 1. More than 83% (170/203) of the *Salmonella* were isolated at the MRC Clinic at Fajara in the Western Region, whereas 16% (33/203) were isolated from the Upper River and Lower River regions (Figure 1). *Salmonella* from the Western Region were isolated from various clinical specimens through passive surveillance from inpatients and outpatients who attended the MRC Clinic between 2005 and 2013. *Salmonella* isolated from blood (n = 106), CSF (n = 1), and aspirates (n = 3) accounted for 54% (110/203) of the isolates. Thirty *Salmonella* isolates from the Upper River Region were cultured from stool specimens collected from children with moderate to severe diarrhea. Three *Salmonella* isolates from the Lower River Region were isolated from the stool of moderately and severely malnourished children aged 9–24 months (n = 3).

**Prevalence of Invasive and Noninvasive *Salmonella***

The prevalence of *Salmonella* in blood was 0.08% (106/13,905) during the 10-year period (Figure 2A). The prevalence of *Salmonella* in blood cultures ranged from 0.4% in 2011 to 1% in 2006, 2007, and 2014 (Figure 2A). Half of invasive *Salmonella* infections occurred between September and November, which mirrors the high malaria transmission season from September to January in The Gambia (Figure 2B).

The prevalence of *Salmonella* in stool among children <5 years old with moderate to severe diarrhea was 2% (39/1958) in the Upper River Region between 2007 and 2012. More than 40% (12/30) of these *Salmonella* infections occurred...
between November and January, which coincides with the peak malaria transmission season in The Gambia. Salmonella were isolated from 2% (2/140) of the stool specimens collected from moderately and severely malnourished children aged 9–24 months in the Lower River Region.

**Phenotypic Characterization of Invasive and Noninvasive Salmonellae**

Of 203 Salmonella isolates, 75% (152/203) were serotyped, and a quarter (51/203) could not be recovered from storage for serotyping. Of the serotyped salmonellae, 14% (21/152) were Salmonella enterica serovar Typhi, whereas 86% (131/152) were serovars other than Typhi (non-typhoidal Salmonella [NTS]). Of the 152 typed NTS isolates, 40% (61) were Salmonella enterica serovar Typhimurium, 10% (10) were Salmonella enterica serovar Enteritidis, and 3% (5) were Salmonella enterica serovar Arizonae. A significantly larger proportion of Salmonella Typhi isolates (70% [16/23]) were invasive compared with 48% (63/131) NTS that were invasive (P = .004; Figure 3). We also identified 3 Salmonella Arizonae infections, of which 1 was from urine, 1 was from stool, and 1 was another BSI in a patient with acute liver disease. Nearly all (90%) of the 10 Salmonella Enteritidis isolates were isolated from blood. Similarly, 80% (33/41) and 76% (16/21) of the Salmonella Typhimurium and Salmonella Typhi isolates, respectively, were from blood. A smaller proportion of untyped NTS isolates were from blood (23% [18/77]). A serogroup C NTS was isolated from a CSF specimen collected from a 4-month-old infant.

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing was performed on all 203 salmonella isolated by disk diffusion method. Susceptibility to ciprofloxacin and to ceftriaxone was 98% (199/203) while susceptibility to gentamicin and chloramphenicol was 95% (193/203) and 94% (194/203), respectively. Susceptibility to each antibiotic (ampicillin, cotrimoxazole, and tetracycline) was 83% (169/203).

Overall, 79% (142/203) of the Salmonella isolates were susceptible to all the antibiotics tested, while the least resistance was to at least 1 antibiotic. Intermediate and full resistance to ≥2 antibiotics was found in 18% (27/203) of the isolates. Intermediate resistance, defined as resistance to ampicillin, cotrimoxazole, and chloramphenicol, was found in 4% (9/203) of the isolates, 3 of which were Salmonella Enteritidis. One untyped NTS isolate from stool was only susceptible to gentamicin and ceftriaxone among the 7 antibiotics tested. Two of the 3 Salmonella Arizonae isolates had intermediate or full resistance to ampicillin, cotrimoxazole, and tetracycline. Susceptibility to each of the 7 antibiotics tested was ≥80% among the 41 Salmonella Typhimurium isolates (Figure 3). In contrast, susceptibility to ampicillin, cotrimoxazole, and chloramphenicol was 60%, 70%, and 79%, respectively, among the 10 Salmonella Enteritidis isolates. Susceptibility to each of the antibiotics tested was ≥80% among Salmonella Typhi and untyped NTS isolates (Figure 3). Between 2005 and 2014, there were differences in the susceptibility to the panel of 7 antibiotics tested. The highest rates of intermediate and full resistance were found in isolates from 2011, whereas the lowest rates were found among 2013 salmonella (Figure 4). Remarkably, all 15 of the 2013 isolates were susceptible to all the antibiotics, and susceptibility to all antibiotics was >90% among all 24 salmonella from 2014.

**Concomitants and Respect of Salmonella Infections**

Nearly half (86/203) of the salmonella isolates were confirmed to be from hospitalized individuals. Noninvasive Salmonella isolates were primarily from stool (85% [59/93]). Other specimens included wounds and abscesses, urine, and an ear swab. Ten patients had 2 or 3 Salmonella isolates from different clinical specimens collected at the same time and/or from the same clinical specimens collected at different times (Table 2). Two
patients had concurrent isolation of salmonellae from stool and blood. Likewise, another patient had concurrent Salmonella infections in urine and blood, while yet another patient had concurrent abscess and bloodstream Salmonella infections (Table 1). Five patients had Salmonella isolated from blood specimens collected several days or months apart. For instance, patient 6 had Salmonella Typhimurium BSI in January, March, and November of the same year; all 3 isolates were pan-susceptible. Patient 4 had a pan-susceptible Salmonella isolate from blood in April and a Salmonella isolate resistant to 4 of the 7 antibiotics tested isolated a month later (Table 2). A 10-month-old infant had Salmonella isolated from CSF 3 days after a Salmonella BSI was found. The records for provisional clinical diagnoses had several gaps for patients from the MRC Clinic in the Western Region, but among the 48 available records for provisional clinical diagnosis, the most common reports were sepsis (22% [11/48]), diarrheal/gastroenteritis (19% [9/48]), and malnutrition (21% [10/48]).

**DISCUSSION**

This study provides data on the trends and phenotypic characteristics of invasive and noninvasive Salmonella in The Gambia, West Africa. Data on the epidemiology and characteristics of salmonellae causing invasive and noninvasive disease is scant in sub-Saharan Africa and even more so in the West Africa subregion [1]. For the first time, this study reveals the role of both S. Typhi and NTS in invasive and noninvasive disease in The Gambia. This study also shows that Salmonella may also be associated with a wide spectrum of infections diseases, including meningitis, enteric infections, abscesses and wound infections, peritonitis, urinary tract infections, and otitis media, which also warrants further investigation. The findings of this study may provide justification for setting up systematic surveillance for both invasive and noninvasive Salmonella infections in this subregion.

In developed countries, NTS are associated with self-limiting diarrheal disease and rarely cause invasive infections such as sepsis [15]. Studies in West Africa and other parts of sub-Saharan Africa have shown that nontyphoidal salmonellae are among the leading causes of invasive disease across all age groups. The prevalence of Salmonella bacteremia in an urban hospital in Ghana was 6.5%, with >60% of the infections attributed to NTS [16]. In a study of invasive bacterial infections and malaria conducted in a rural hospital and health center in Burkina Faso, the prevalence of salmonellae in blood cultures was
Among blood cultures from 871 patients who attended the MRC clinic between 2003 and 2005, the prevalence of NTS was 9.9% and that of Salmonella Typhi was 0.1% [9]. In the 10 years covered by this study, the prevalence of salmonellae in BSIs infections was 0.7%, slightly below what was previously reported [9, 10]. The prevalence of salmonellae in BSIs varied between 0.5% and 1% over the 10 years covered in this study. This study also confirms that Salmonella Typhi, although less prevalent than NTS, continues to cause BSIs in The Gambia.

Nearly half of the Salmonella infections found were in children <5 years old, and a third of the patients were between 15 and 60 years old. This is consistent with previous studies in The Gambia which showed that Salmonella is an important cause of invasive bacteremia across all age groups in this setting [8–11, 18]. From 2005 to 2015, 85% and 99% of Salmonella Typhimurium and Salmonella Enteritidis isolates, respectively, were isolated from blood specimens. In sharp contrast, less than a quarter of untyped NTS isolates were from blood specimens. This suggests that the largest burden of invasive NTS infections may be attributable to Salmonella Typhimurium and Salmonella Enteritidis in The Gambia.

In a study on community-acquired invasive NTS infections among children 2–29 months old conducted in the Upper River Region in 2006, Ilumapay and colleagues found that 88% of all invasive NTS infections were attributed to Salmonella Enteritidis and Salmonella Typhimurium [19]. However, in contrast to the current study, where there were nearly 2-fold more Salmonella Typhimurium than Salmonella Enteritidis invasive infections, Ilumapay and colleagues found that Salmonella Enteritidis invasive infections were 10 times more frequent than those caused by Salmonella Typhimurium [10]. A study conducted in a tertiary care setting between 2010 and 2013 in Ghana showed that 80% and 20% of NTS BSIs were attributed to Salmonella Enteritidis and Salmonella Typhimurium, respectively. Although data on the prevalence of NTS in sub-Saharan Africa is scarce, there is evidence that there are regional, temporal, and seasonal differences in the circulating Salmonella serovars [3, 19].

Salmonella invasive infections are associated with high case fatality rates (>20%), and rapid treatment with the appropriate antimicrobial therapy is vital [10, 16, 20–22]. As previously reported in The Gambia, antibiotic susceptibility among invasive salmonellae is >80% for ampicillin, tetracycline, ceftriaxone, chloramphenicol, ciprofloxacin, and co-trimoxazole. This is in sharp contrast to reports from Ghana, Burkina Faso, and other parts of sub-Saharan Africa where resistance to ampicillin, tetracycline, and chloramphenicol can exceed 70% in invasive salmonellae [16, 17, 23, 24]. The highest rates of intermediate resistance and resistance were 19%–23% against ampicillin and ceftriaxone, which were previously first-line treatments for invasive and enteric NTS infections [3]. Due to increasing prevalence of...
Table 2. Characteristics of Patients From Whom Salmonella Was Isolated From Different Sources and/or at Different Times in The Gambia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, y</th>
<th>Date (Day/Month/Year)</th>
<th>CSF</th>
<th>Blood</th>
<th>Abscess</th>
<th>Urine</th>
<th>Stool</th>
<th>Isolate</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1*</td>
<td>Female</td>
<td>1</td>
<td>10/06/2005</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Typhimurium</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Female</td>
<td>10</td>
<td>19/01/2006</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Salmonella</td>
<td>Amp: S, S, S, R, S, S, S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>04/01/2000</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>Female</td>
<td>25</td>
<td>24/04/2006</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>Salmonella</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26/05/2006</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>Female</td>
<td>27</td>
<td>16/06/2007</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Typhi</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td>Patient 6</td>
<td>Male</td>
<td>33</td>
<td>04/01/2006</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Typhimurium</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27/03/2006</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 7</td>
<td>Female</td>
<td>32</td>
<td>13/05/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Enteritidis</td>
<td>Amp: R, S, S, S, S, S, R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>03/06/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 8</td>
<td>Male</td>
<td>0.8</td>
<td>08/03/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Typhimurium</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12/03/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 9</td>
<td>Female</td>
<td>0.8</td>
<td>06/10/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Salmonella Typhimurium</td>
<td>Amp: S, S, S, S, S, S, S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15/10/2009</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23/02/2006</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Amp, ampicillin; Chl, chloramphenicol; Cip, ciprofloxacin; Chn, gentamicin; Cro, ceftriaxone; CSF, cerebrospinal fluid; Sxt, cotrimoxazole; Te, tetracycline.

* Salmonella was isolated from 2 blood samples collected from patient 1 at 2 different times on the same day.
resistance, third-generation cephalosporins and fluorquinolones are recommended for the treatment of NTS infections [3]. It is reassuring that nearly all the salmonellae were susceptible to chloramphenicol, cephalothin, and cotrimoxazole. However, the finding that 9 (9%) of the 203 Salmonella isolates were MDR is of great concern. MDR salmonellae may be associated with higher morbidity and pose a major public health concern in sub-Saharan Africa that requires continuous monitoring [3]. Although overall prevalence of resistance was low, nearly one-fifth of the isolates were not susceptible to at least 2 of the antibiotics tested and more than one-tenth were not susceptible to at least 3 antibiotics. One NTS isolate from stool was only susceptible to gentamicin and ceftriaxone of the 7 antibiotics tested. The presence, albeit low, of MDR Salmonella warrants continued surveillance and monitoring in The Gambia and sub-region.

Interestingly, resistance to ampicillin and cotrimoxazole was at least 3-fold more prevalent in Salmonella Enteritidis compared with Salmonella Typhimurium isolates. Although a third of the Salmonella Enteritidis strains were resistant to chloramphenicol, all the Salmonella Typhimurium isolates were susceptible to this antibiotic (Figure 2). Furthermore, a third of the Salmonella Enteritidis isolates were MDR. Serovar differences in antibiotic susceptibility patterns have been reported previously in The Gambia, as have higher rates of antimicrobial resistance in Salmonella Enteritidis isolates [10].

We also identified 3 Salmonella Arizonae infections, of which 1 was a primary trach infection and another a BSI in a patient with suspected acute liver disease. A serogroup B NTS was found in the CSF and blood of a 4-month-old infant. Although rare, cases of Salmonella bacterial meningitis have been reported elsewhere in Africa and are often associated with high case fatality rates [24-28]. At least 5 patients had Salmonella isolated from blood specimens at intervals spanning several days and months (Table 2). In one instance, the second isolate had a distinct antimicrobial resistance pattern from the first. Whole-genome sequencing can be used to establish if these are congenital reinfections or relapse [29]. This study has several limitations in design and laboratory methods. It is a retrospective study with large gaps in the metadata and clinical data, particularly for isolates from the MRG Clinic. All the invasive isolates were from the Western Region, and this may mask geographic differences in the salmonellae causing disease in The Gambia. Furthermore, 52 isolates were not serotyped to NTS or Salmonella Typhi, which means the actual prevalence of Salmonella Typhi may be underestimated in this study. Likewise, a large proportion of NTS isolates were not serotyped, which could also have skewed the prevalence data presented here. The disk diffusion method was used to determine susceptibility, and minimum inhibitory concentrations were not used to confirm resistance, except for chloramphenicol and tetracycline. However, we previously showed that the disk diffusion susceptibilities for salmonellae are insensitive and reliable for guiding treatment [10].

This retrospective study took advantage of archived salmonellae covering a 10-year period. This study, despite its limitations, provides some important insights into the phenotypic characteristics and antibiotic resistance patterns of salmonellae associated with invasive and noninvasive infections in this part of West Africa. The data presented here provide 10-year baseline data and justification for continuous surveillance of Salmonella infections, antibiotic resistance, and possible vaccine trials in the future.

Notes
Acknowledgements. We would like to thank Yemani Gambia and Richard Adegbola for collecting and storing the clinical specimens and microbiology data prior to 2010 from Falaka. Richard Adegbola provided advice on the manuscript, and all the patients and individuals who participated in the study.

Author contributions. B.K.A., S.D., and M.A. wrote the manuscript. B.K.A. prepared the figures and tables. All authors reviewed and contributed to the final manuscript.

Disclaimer. The Medical Research Council (MRC) Unit in The Gambia and the Bill & Melinda Gates Foundation did not participate in the study design, the collection, analysis, and interpretation of data or the writing of the report.

Financial support. This work was supported by MRC (UK) funds.

Supplement sponsorship. This article appeared as part of the supplement “Invasive Salmonella 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


