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Antimicrobial resistance and virulence genes of invasive *Salmonella enterica* from children with bacteremia in north-central Nigeria

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Abstract

Objectives: Bacteremia due to invasive *Salmonella enterica* has been reported earlier in children in Nigeria. This study aimed to detect the virulence and antibiotic resistance genes of invasive *Salmonella enterica* from children with bacteremia in north-central Nigeria.

Method: From June 2015 to June 2018, 4163 blood cultures yielded 83 *Salmonella* isolates. This is a secondary cross-sectional analysis of the *Salmonella* isolates. The *Salmonella enterica* were isolated and identified using standard bacteriology protocol. Biochemical identifications of the *Salmonella enterica* were made by Phoenix MD 50 identification system. Further identification and confirmation were done with polyvalent antisera O and *inv*A gene. Antimicrobial susceptibility testing was done following clinical and laboratory standard institute guidelines. Resistant genes and virulence genes were determined using a real-time polymerase chain reaction.

Result: *Salmonella typhi* 51 (61.4%) was the most prevalent serovar, followed by *Salmonella* species 13 (15.7%), *choleraesuis* 8 (9.6%), *enteritidis* 6 (7.2%), and *typhimurium* 5 (6.1%). Fifty-one (61.4%) of 83 *Salmonella enterica* were typhoidal, while 32 (38.6%) were not. Sixty-five (78.3%) of the 83 *Salmonella enterica* isolates were resistant to ampicillin and trimethoprim-sulfamethoxazole, followed by chloramphenicol 39 (46.7%), tetracycline 41 (41.4%), piperacillin 33 (33.9%), amoxicillin-clavulanate, and streptomycin 21 (25.3%), while cephalothin was 19 (22.9%). Thirty-nine (46.9%) of the 83 *Salmonella enterica* isolates were multi-drug resistant, and none were extensive drug resistant or pan-drug resistant. A bla_{TEM} 42 (50.6%), *flo*R 32 (38.6%), *qnr*A 24 (28.9%), *tet*B 20 (20.1%), *tet*A 10 (10.0%), and *tet*G 5 (6.0%) were the antibiotic resistance genes detected. There were perfect agreement between phenotypic and genotypic detection of antimicrobial resistance in tetracycline, ciprofloxacin, and chloramphenicol, while beta-lactam showed $\kappa = 0.60$ agreement. All of the *Salmonella enterica* isolates had the virulence genes *inv*A, *sop*B, *mgt*C, and *sip*4D, while 33 (39.8%), 45 (51.8%), and 2 (2.4%) had *ssa*Q, *spv*C, and *ljs*GI-1, respectively.

Conclusion: Our findings showed multi-drug resistant *Salmonella enterica* in children with bacteremia in northern Nigeria. In addition, significant virulence and antimicrobial resistance genes were found in invasive *Salmonella enterica* in northern Nigeria. Thus, our study emphasizes the need to monitor antimicrobial resistance in *Salmonella enterica* from invasive sources in Nigeria and supports antibiotic prudence.

Keywords

Salmonella typhi, non-typhoidal *Salmonella*, northern Nigeria, antibiotic resistance, bacteremia

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Introduction

The type of infection that results from *Salmonella enterica* is determined by the virulence factors of the bacterium and the host's factors.[1–3] *Salmonella* infection could manifest clinically as gastroenteritis (diarrhea, abdominal cramps, and fever) or a fatal febrile systemic infection (typhoid) that needs to be treated with antibiotics.[4–7] Focal conditions and asymptomatic carriers are possible and are significant sources of continued infection transmission.[8] *Salmonella* is a gram-negative, flagellated, with O, H, and Vi antigens. More than 1800 *Salmonella* serovars are known.[2,9] *Salmonellae* infection is possible when the bacteria gets through the gastric acid barrier, into the mucosa of the small and large intestines, and makes toxins.[10] Invasion of the epithelial cells causes the release of cytokines that cause inflammation.[11] It results in diarrhea, leading to ulceration and the destruction of mucosal cells. Also, if this spread in the intestines persists, it could result in systemic infection.[11]

Horizontal gene transfers shape bacterial genomic diversity.[12] Some pathogens have genomic islands or islets (GI) with functionally linked genes.[13,14] *Salmonella* pathogenic islands (SPIs) contain virulence genes, distinguishing them from nonpathogenic types.[15–18] Virulence factors determine a host's pathogenicity.[19] The adhesion and invasion of host cells by pathogenic *S. enterica* are aided by *pef*, *spv*, *inv*A, and *fim* genes, respectively. Magnesium transport C (mgtC), *Salmonella* toxin (*stn*), and *pip A*, *B*, and *D* help the bacterium survive in the host system.[20,21]

The burden of invasive bloodstream infection due to *S. enterica* is increasing, especially in developing countries.[5] Globally, 1.2 million deaths attributable to *S. enterica* are recorded annually, with the vast majority occurring in resource-limited countries.[22] In resource-limited countries, non-typhoidal *Salmonella* infections cause bacteremia in immune-compromised and malnourished adults and children.[16] Most morality from *Salmonella* infection is connected to poor diagnostic infrastructures leading to misdiagnosis and drug misuse.[23] It is established that most pathogenic bacteria are acquired from the environment, food, and water sources.[24,25] Although typhoidal *Salmonella* is a human host-adapted strain, recent literature has found typhoidal *Salmonella* in the food chain and water sources.[26–30]

The worldwide rise of multi-drug resistance is a major health concern.[31] It is becoming increasingly important to routinely apply antimicrobial susceptibility testing to select the antibiotic of choice and to screen for emerging multi-drug resistant (MDR) strains,[32] as several recent studies have reported the emergence of multi-drug resistant *Salmonella* pathogens from various origins, including humans,[33] birds,[34] cattle,[31] and fish.[35]

Pathogens' high antimicrobial resistance has been attributed to antimicrobial misuse in human and animal husbandry.[36–39] Comparative genomic data from invasive *Salmonella* data and those from the environment and food chain have shown relatedness between clinical isolates and other sources.[10,11,40] It has caused great concern as clinical *S. enterica* are resistant to the commonly used antibiotic, and some have been found to harbor extended-spectrum beta-lactamases (ESBL) genes which could make treatment difficult.[41–46] Recent data from surveillance has found non-typhoidal *Salmonellae* to be highly resistant to antimicrobials.[4,47–49] Thus, clinical care for individuals with invasive *Salmonella* infection is expensive, increases their hospital stay, and burdens them financially.[50,51] Studies have been carried out on *Salmonella* virulence factors, but information on invasive isolates is scarce, especially from the pediatric population. This study aims to investigate the virulence and antimicrobial resistance genes pattern of *S. enterica* from invasive bloodstream infection in children from north-central Nigeria.

Materials and methods

Study design

This is a secondary cross-sectional analysis of isolated *S. enterica* in children with bloodstream infection in the Federal Capital Territory (FCT) and Nasarawa State, Nigeria.

Collection of Salmonella *isolates analyzed in the study*

Eighty-three gram-negative bacilli isolates were collected from blood cultures recovered from the study conducted at seven hospitals in FCT and Nasarawa State, Nigeria. Presumptively identified *Salmonella* isolates from four thousand and sixteen blood cultures were processed from June 2015 to June 2018. The study was part of Community-Acquired Bacteremia Syndrome in Young Nigeria Children conducted in north-central Nigeria from 2008 to 2018 by the International Foundation Against Infectious Diseases in Nigeria. The outcomes from 2008 to 2015 had been previously reported by Obaro et al.,[5] and those previously reported isolates were excluded from this study.

Isolation of S. enterica *of positive blood culture*

Bacterial analysis, including gram staining and biochemical analysis using the analytical profile index (API20E) (Biomerieux, SA Lyon, France), was used to identify the *Salmonella* pathogens. Obaro et al.[5] have previously described the protocol used to culture and isolate the *S. enterica* used in this work. Briefly, all positive bottles were subcultured onto MacConkey agar (Oxoid, London, UK) and *Salmonella shigella* agar (Oxoid) plates and then incubated at 36°C for 24 h. The isolates were frozen at −80°C in 10% skim milk glycerol (Hardy Diagnostics, Santa Maria, California, USA) until used.[52] In conducting the study, the previously collected isolates were grown onto *S. shigella*

agar (Oxoid) and incubated at 37°C for 24 h. In checking for *Salmonella* isolates, colonies were looked for on the plates. The morphological traits and characteristics of *Salmonella* species were selected, and gram staining of the selected colonies from each plate was examined.[52] Biochemical assays, including reactions on triple sugar iron agar, lysine iron agar, indole synthesis in tryptone broth, and urea splitting ability, were then conducted using the Phoenix MD (Beckon-Dickson systems, San Jose, California, USA). Molecular *inv*A gene detection was used to validate the authenticity of the isolates. Polyvalent *Salmonella* antisera, A-G, A-S surface antigen, flagellar H (phase 1 and phase 2) (Beckon-Dickson systems) according to Kauffman-White Scheme[53] were used in the serotyping of the *Salmonella* isolates.

By Antimicrobial Susceptibility Testing and Multiple Antimicrobial Resistance (MAR) Index according to Clinical and Laboratory Standards Institute recommendations,[54] the antibiotic susceptibility of the isolates was determined. The antibiotic discs (ampicillin (10 µg), amoxicillin-clavulanate (30/10 µg), piperacillin (30 µg), piperacillin-tazobactam (30/10 µg), streptomycin (10 µg), trimethoprim-sulfamethoxazole (10/25 µg), chloramphenicol (30 µg), tetracycline (30 µg), aztreonam (30 µg), gentamicin (10 µg), amikacin (30 µg), cephalothin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), ceftriaxone (30 µg), levofloxacin (5 µg), meropenem (10 µg), imipenem (10 µg), tigecycline (25 µg), cefotaxime-clavulanate (30/10 µg), and ceftazidime-clavulanate (30/10 µg) that were utilized in a disk diffusion assay were from Oxoid. The BD Phoenix™ M50 system (Beckon-Dickson systems) was used for minimal inhibitory concentration (MIC) testing. For ciprofloxacin, MIC values >0.064 g/mL were viewed as reduced susceptibility, while MIC values 4 g/mL were interpreted as resistant; for azithromycin, MIC values >16 g/mL were interpreted as resistant. According to Davis and Brown,[55] the MAR indexes were derived as the ratio of antibiotics to which resistance was demonstrated to the number of antibiotics for which the isolate was screened for susceptibility. According to Algammal et al.,[32] the resistance profiles were classified as MDR, extensive drug resistant (XDR), or pan-drug resistant (PDR).

Molecular detection of resistance and virulence genes

Genomic DNA was extracted using the Maxwell 16-cell DNA purification kit (Promega, Madison, Wisconsin, USA) on an automated machine (Maxwell 16 extraction system, Madison, Wisconsin, USA). The real-time polymerase chain reaction (PCR) assays were performed on the AriaMx system (Agilent Inc., Santa Clara, California, USA). Primers and probes were purchased from LGC, Biosearch (Novato, California, USA) for the different genes based on primers and probes used by Ibrahim et al.[56] for *inv*A; Bugarel Weil et al.[57] for sopB, ssaQ, mgtC, spi4D, spvC, and ljsGI-1; Roschanski et al.[58] for bla_{TEM}, bla_{SHV}; Vien et al.[59] for *qnr*A; Singh and Mustapha[60] for *flo*R and *tet*G; and Guarddon et al.[61] for *tet*A and *tet*B. Supplemental Tables 1 and 2 show the genes sequences and amplification conditions used. A quality-controlled positive and negative internally characterized known resistant and susceptible *Salmonella typhi* strains from International Typhoid Consortium[62] were used as controls for amplification for detecting the resistance genes and virulence during PCR. Also, no template controls were incorporated into the PCR as an additional method of internal control in the PCR.

Table 1. Identification of *Salmonella* by API 20E.

Salmonella ID	Clinical samples *n* = 83 *n* (%)
Salmonella typhi	51 (61.4)
Salmonella typhimurium	5 (6.1)
Salmonella enteritidis	6 (7.2)
Salmonella choleraesuis	8 (9.6)
Salmonella species	13 (15.7)

n: number; %: percentage.

A 12.5 µL of Perfecta master mix low ROX kit (Quanta Bioscience Inc., Madison, Wisconsin, USA), 1.0 µL of each 10 mM primers and probes, 7.5 µL of Nuclease free water (Sigma-Aldrich, St Louis, Missouri, USA), and 2.0 µL of DNA template make up a 25 µL PCR reaction mixture. Thermal conditions were those described by the referenced authors (Tables 1 and 2). After the amplification experiments were completed, the cycle thresholds were determined by identifying the fluorescence signal by analyzing the amplification plots in AriaMx system software version 3.1.

Statistical analysis

Data were imputed and validated in Excel 2016. Descriptive statistics were computed for the multiple antibiotic resistance index. Agreement between the values of antimicrobial resistance phenotypes and their corresponding genotypes was established by κ value (coefficient of agreement) according to Jeamsripong et al.[63] Chi-square and Fisher's exact test were used to test association as appropriate in every case. $p < 0.05$ was taken as statistically significant. Statistical Package for Social Science Version 20 (IBM, Santa Barbara, California, USA) was used.

Results

Prevalence and phenotypic characteristics of recovered Salmonella *species*

Table 1 shows the *Salmonella* serovars found in the study, *S. typhi* 51 (61.4%) was the most occurring serovar, *Salmonella typhimurium* 5 (6.1%), *Salmonella enteritidis* 6 (7.2%), *Salmonella choleraesuis* 8 (9.6%), and *Salmonella* species

Table 2. The non-susceptibility pattern between invasive typhoidal and non-typhoidal *Salmonellae* in the study.

Classes	Antibiotics	No of positive isolates $n=83$ (%)	*Salmonella enterica*		*p*-Value
			Typhoidal $n=51$	Non-typhoidal $n=32$	
Beta-lactams	Ampicillin*	65 (78.3)	44 (86.3)	21 (65.6)	0.03
Penicillin	Amoxicillin-clavulanate*	21 (25.3)	13 (25.5)	9 (28.1)	0.79
First cephalosporin	Piperacillin*	33 (39.8)	20 (39.2)	13 (40.6)	0.41
	Cephalothin*	19 (22.9)	12 (23.5)	7 (21.9)	0.86
Sulfonamides	Trimethoprim-sulfamethoxazole*	65 (78.3)	44 (86.3)	21 (65.6)	0.03
Phenicol	Chloramphenicol*	39 (46.9)	22 (43.1)	17 (53.1)	0.37
Tetracycline	Tetracycline*	41 (49.4)	33 (64.7)	8 (25.0)	0.0004
Aminoglycosides	Streptomycin*	21 (25.3)	10 (19.6)	11 (34.4)	0.13
	[a]Azithromycin (IAS)**	9 (10.8)	5 (9.8)	4 (12.5)	0.73
Fluoroquinolone	[b]Ciprofloxacin (ICS)*	24 (28.9)	16 (31.4)	8 (25.0)	0.53

n: number; %: percentage; ICS: intermediate ciprofloxacin susceptibility; IAS: intermediate azithromycin susceptibility. $p<0.05$ = statistical significance. $p>0.05$ = statistical insignificance.
*Chi-square statistic.
**Fisher's exact test.
[a]MIC ciprofloxacin >0.064 µg/mL.
[b]MIC azithromycin >16 µg/mL.

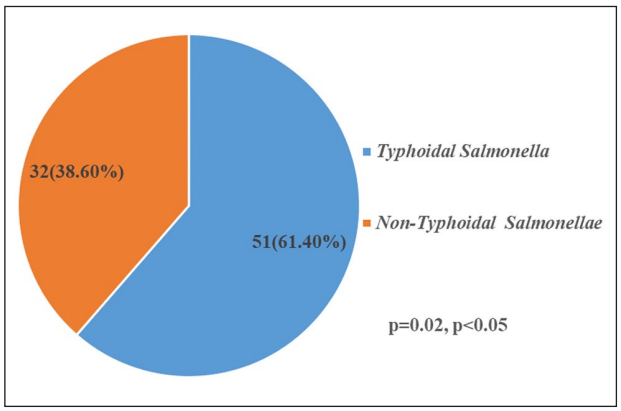

Figure 1. Classification of *Salmonella enterica* in the study.

13 (15.7%) were the other identified serovars in this study. Figure 1 showed a statistically significant ($p=0.02$) higher typhoidal *Salmonellae* 51 (61.4%) than non-typhoidal 32 (38.6%) *Salmonellae* in the study.

Antimicrobial susceptibility testing, antibiotic resistance genes outcomes

Table 2 shows the resistance pattern of the *Salmonella* isolates. Of the 83 *S. enterica* isolates, 65 (78.3%) were resistant to ampicillin and trimethoprim-sulfamethoxazole. A significant (p = 0.03) higher resistance was found in typhoidal Salmonellae 44 (86.3%) compared non-typhoidal Salmonellae 21 (65.6%). Resistance to tetracycline 41 (49.4%) was higher, with a statistically significant difference ($p=0.0004$) in typhoidal *Salmonellae* 33 (64.7%) than in non-typhoidal *Salmonellae* 8 (25.0%).

Resistance to other antimicrobials was as follows: amoxicillin-clavulanate 21 (25.3%), piperacillin 33 (39.8%), cephalothin 19 (22.9%), chloramphenicol 39 (46.9%), streptomycin 21 (25.3%), azithromycin 9 (10.8%) with no statistically significant differences ($p>0.05$) in typhoidal *Salmonellae* and non-typhoidal *Salmonella* in the study. Intermediate ciprofloxacin susceptibility occurred in 24 (28.9%) *Salmonella* isolates. Also, intermediate azithromycin susceptibility occurred in nine (10.8%) *Salmonella* isolates.

MDR was found in 39 (46.9%) of the 83 *S. enterica* isolated. Of the 39 *S. enterica* isolates that demonstrated MDR phenotypically, 30 (58.9%) were typhoidal *Salmonellae*. In comparison, nine (28.1%) of them were non-typhoidal *Salmonellae*. No XDR and PDR were observed in the study, as shown in Figure 2. Figure 3 shows the MAR indexes of the *Salmonella* isolates, 50 (60.2%) of the isolates showed higher statistically significant MAR (>0.2) than the other 33 (39.8%) *Salmonella* isolates with MAR ⩽ 0.2.

Of the resistance genes investigated, the bla_{TEM} gene with 42 (50.5%) was the most common occurrence in the study. The occurrences of the other resistance genes were as follows, *flo*R 32 (38.6%), *tet*A 10 (12.0%), *tet*B 20 (24.1%), *tet*G 5 (6.0%), and *qnr*A genes 24 (28.9%). The study showed no bla_{SHV} and bla_{CTX-M}, as shown in Figure 4. Table 3 shows the occurrence of the resistance genes in typhoidal and non-typhoidal *S. enterica*. There was no statistical significance ($p>0.05$) differences in the occurrence of the resistance genes in typhoidal and non-typhoidal *S. enterica*.

For even phenotypic resistance recorded, the corresponding gene was determined, κ agreement analysis was done, and the outcomes showed perfect agreement for chloramphenicol ($\kappa=0.954$), tetracycline ($\kappa=1$), and ciprofloxacin

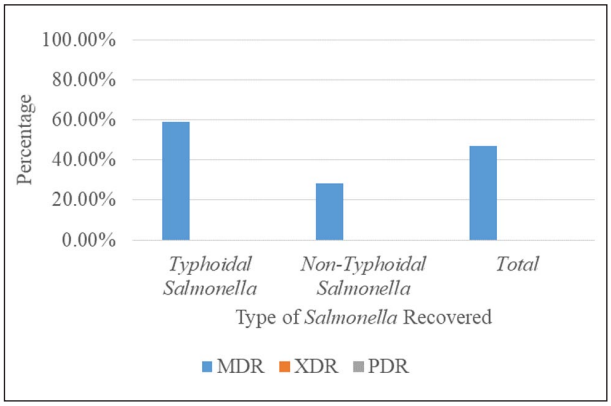

Figure 2. Occurrence of MDR of *Salmonella enterica* isolated.
MDR: multi-drug resistant.

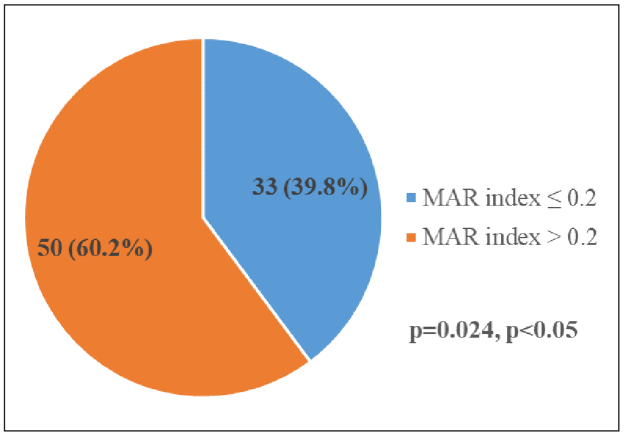

Figure 3. Multiple antimicrobial resistance index of *Salmonella* serovars.

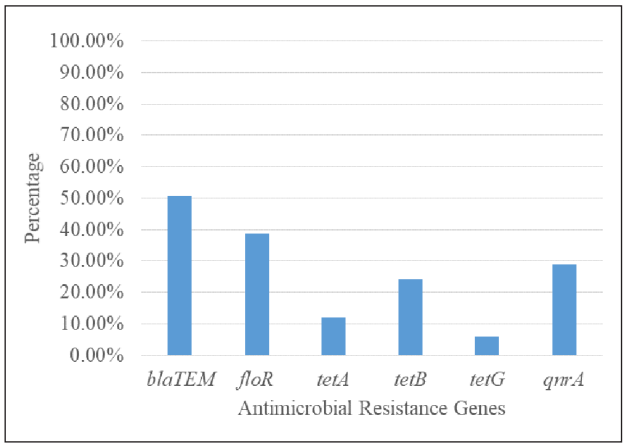

Figure 4. Prevalence of resistance genes from the *Salmonella enterica* isolates.

(κ = 1), but there was a moderate agreement between phenotypic detection of β-lactam and the genotypic detection with κ = 0.60 as shown in Supplemental Table 3.

PCR-based detection of virulence-determined genes

Of the seven virulence genes examined, SPIs encoding genes (*inv*A, *sop*B, *mgt*C, and *spi*4D) were found in all the *Salmonella* isolates 83 (100.0%). Gene *spv*C occurred in 45 (51.8%) *S. enterica* recovered in the study. In comparison, gene *ssa*Q occurrence was found in 33 (39.8 %) of the *Salmonella* isolates, but the *ljs*GI-1 gene was found in 2 (2.4%) of the *Salmonella* isolates shown in Figure 5. The prevalence of *spv*C genes in typhoidal *Salmonellae* 30 (58.8%) was insignificantly higher than non-typhoidal *Salmonellae* 16 (57.1%). In contrast, gene *ssa*Q occurrence in typhoidal *Salmonellae* 17 (33.3%) is significantly ($p = 0.02$) lower than in non-typhoidal *Salmonellae* 16 (57.1%). The *ljs*GI-1 gene was found only in two (3.9%) typhoidal sub-group, as shown in Table 4.

Discussion

Increased resistant *S. enterica* has continued to pose a significant threat to human health and animal protection, and their spread is being increasingly found in clinical, food, and animal samples. Our study found typhoidal *Salmonella* serovars and non-typhoidal *Salmonella* serovars similar to the report by Awol et al.[64] in a multicenter study. Ke et al.[65] and Stanaway et al.[66] show that children in poor and middle-income countries with sub-optimal water, sanitation, and hygiene have a considerable and progressive increase in invasive non-typhoidal *Salmonella* (iNTS) infection. Invasive typhoid and non-typhoidal *Salmonella* have been previously reported in Nigerian children.[5,8,27,67,68]

Regarding the serovars found in this study, *S. typhi* was the highest, followed by *S. choleraesuis*, *S. enteritidis*, and *S. typhimurium. Salmonella typhi* is the most common serovar in invasive *Salmonella* infection in children, according to previous studies conducted in Nigeria and elsewhere.[5,8,67,69–71] Serovars *S. enteritidis and S. typhimurium* are not frequently observed in invasive non-typhoidal infections in industrialized countries. Still, in sub-Saharan Africa, they are becoming a reoccurring decimal. Two African authors have previously reported them in invasive *Salmonella* infection.[72,73]

High levels of resistance to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and other routinely used antibiotics were found in our investigation. *Salmonella* strains isolated from invasive environments resist many of the most widely used antibiotics.[5,67,70,71] In particular, multidrug-resistant iNTS caused life-threatening invasive disease outbreaks in children in Nigeria, Rwanda, and Malawi.[67,74,75]

The antimicrobial resistance of iNTS is a big problem because it can cause bacteremia in immunocompromised people.[76] The high prevalence of antimicrobial-resistant *Salmonella* is a serious concern for public health.[58] *Salmonella typhi* was the most common cause of invasive typhoidal

Table 3. Occurrences of resistance genes in typhoidal and non-typhoidal *Salmonella* in the study.

Classes	Genes	No positive isolates $N = 83$ (%)	*Salmonella enterica*		*p*-Value
			Typhoidal $n = 51$	Non-typhoidal $n = 32$	
Beta-lactams	bla_{TEM}*	42 (50.6)	29 (56.9)	13 (40.6)	0.15
	bla_{SHV}	0 (0.0)	0 (0.0)	0 (0.0)	NA
	bla_{CTX-M}	0 (0.0)	0 (0.0)	0 (0.0)	NA
Chloramphenicol	*flo*R*	32 (38.6)	20 (39.2)	12 (37.5)	0.88
Tetracycline	*tet*A**	10 (12.0)	8 (15.7)	2 (6.3)	0.30
	*tet*B*	20 (24.1)	13 (25.5)	7 (21.9)	0.71
	*tet*G**	5 (6.0)	2 (3.9)	3 (9.4)	0.37
Fluoroquinolone	*qnr*A*	24 (28.9)	16 (31.4)	8 (25.0)	0.53

n: number; %: percentage; NA: not applicable. $p < 0.05$ = statistical significance; $p > 0.05$ = statistical insignificance.
*Chi-square statistic.
**Fisher's exact test.

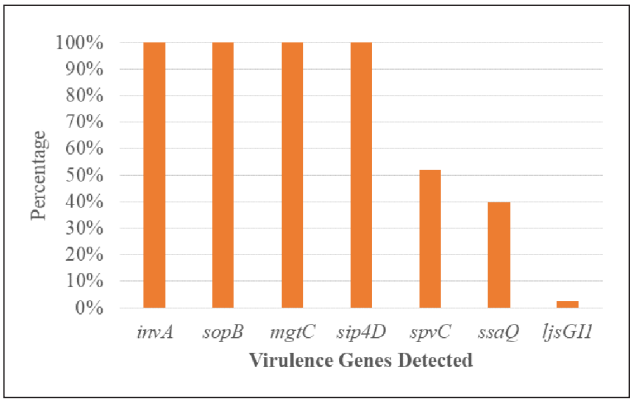

Figure 5. Prevalence of virulence genes from the *Salmonella enterica* isolates.

Table 4. Occurrence of virulence genes in invasive typhoidal and non-typhoidal *Salmonella*.

Genes	Total	Typhoidal	Non-typhoidal	*p*-Value
	$n = 83$ *n* (%)	$n = 51$ *n* (%)	$n = 32$ *n* (%)	
*inv*A	83 (100.0)	51 (100.0)	32 (100.0)	1.0
*sop*B	83 (100.0)	51 (100.0)	32 (100.0)	1.0
*mgt*C	83 (100.0)	51 (100.0)	32 (100.0)	1.0
*Sip*4D	83 (100.0)	51 (100.0)	32 (100.0)	1.0
*spv*C	45 (51.8)	30 (58.8)	15 (42.8)	0.29
*ssa*Q	33 (39.8)	17 (33.3)	16 (57.1)	0.02
*ljs*GI-1	2 (2.4)	2 (3.9)	0 (0.0)	0.52

n: number; %: percentage. $p < 0.05$ = statistical significance; $p > 0.05$ = statistical insignificance.
*Chi-square statistic.
**Fisher's exact test..

Salmonella infection, and its high MDR and low ciprofloxacin susceptibility rates were the most striking findings of our investigation. This result is consistent with patterns seen in Cambodia by Vlieghe et al.[77] when describing MDR in *S. typhi*. The MDR has been observed in *S. enterica* in several countries in literature.[78–80] *Salmonella typhi* with multi-drug resistance was found in an assessment of typhoid fever cases in Pakistan, Vietnam, India, China, Indonesia, and Nigeria.[73,81–85]Our finding regarding MDR in *Salmonella* isolates in Nigeria agreed with previous reports and the assertion that Nigeria is in the vicinity region referred to as a hotspot for antimicrobial overuse.[19,86]

The MAR index, a cost-effective and valid method of bacteria origin tracking, was also calculated. It has become possible to tell bacteria apart by their resistance to the most popular antibiotics in human medicine by doing a MAR analysis.[87–89] Compared to other methods of bacteria source tracking, such as genotypic characterization, the MAR indexing method is cost-effective, rapid, and easy to perform.[90] MAR index values greater than 0.2 indicate a high-risk source of contamination and index for high antibiotics usage.[88] This study reported a high MAR index >0.2 for *S. enterica* from invasive sources, which concerns the efficacy of treatment options available. High MAR in *S. enterica* has been attributed to plasmids containing one or more resistance genes,[91–93] each encoding a single antibiotic resistance phenotype.[94] This study did not find XDR and PDR support in literature.[91–93]

Our results revealed significant positivity of bla_{TEM} in the *S. enterica* studied. The bla_{TEM} gene found in this study was slightly higher in invasive typhoidal strains than iNTS. The presence of the bla_{TEM} gene in most of the *S. enterica* in our study supported earlier assertion, which suggested that bla_{TEM} genes code for beta-lactam drug resistance like ampicillin.[95] Beta-lactamase produced by gram-negative bacteria remains the primary mechanism by which they develop resistance to beta-lactam antibiotics. Additionally, ESBLs are increasingly common among *S. enterica* serovars, and their frequency and prevalence have been reported to rise.[96–98] Although bla_{CTX-M} and bla_{SHV} were not found in this study, recent studies from Asia and some parts of Africa have

reported them,[96,99] specifically reports of the bla_{SHV} gene from clinical *S. enterica* from India.[100–103] Therefore, monitoring the incidence of bla_{TEM} in *S. enterica* isolates is a crucial public health tool in combatting this threat.

Salmonella enterica isolates with phenotypically intermediate ciprofloxacin susceptibility (ICS) were found to harbor plasmid-mediated quinolone resistance (PMQR) genes (*qnr*A). This observation agrees with a report from India[104] with observations in human samples (51.4%), food-producing animals (28.6%), environmental samples (11.4%), and animal samples (8.6%), respectively.[89,105–107] Ciprofloxacin has become prominent in treating severe infections caused by *S. enterica*, especially those resistant to nalidixic acid, which has increased significantly in recent years. Still, high resistance levels to ciprofloxacin are rare, but its resistance is foundational for other resistance mechanisms.[75,108,109] Mutants resistant to fluoroquinolones are being rapidly selected due to the spread of PMQR genes.[110] Moreover, interactions between mutations in the QRDR and PMQR genes might result in high fluoroquinolones MIC. However, a study[111] speculated that the *qnr* genes could increase fluoroquinolone resistance.

In literature, *tet*A, *tet*B, and *tet*G were consistently found in *S. enterica* of human origin. The *tet*B gene is predominant among the phenotypic tetracycline-resistant strains in the literature. Our findings agreed with two studies that have reported *tet*A and *tet*B in *S. enterica* from a human with gastroenteritis in India and Nigeria.[112,113] *tet*G has also been reported in humans with bacteremia in Nigeria.[113] However, studies examining tetracycline resistance in multiple isolates reported *tet*A, *tet*B, and other types in *S. enterica* isolates from humans and those from animals, environments, and poultries.[113,114] Regarding the family *Enterobacteriaceae*, the *tet*B and *tet*A tetracycline resistance determinants have historically been the most prevalent.[112] However, *tet* (C, D, E, M, O) associated with tetracycline resistance in *Salmonella* species and other bacteria are less frequently found.

Salmonella isolates that have developed phenotypic resistance to chloramphenicol are strongly linked to the development and expression of efflux that pumps the drug out of the bacteria's cells,[115,116] encoded by *flo*R or *cml* genes. In our study, all invasive *S. enterica* phenotypically resistant to chloramphenicol had *flo*R gene. These findings agree with reports of floR gene detection from *S. enterica* in the literature.[117,118] Also, it has been asserted that the *flo*R gene of *Salmonella* pathogenicity island-1 contributes to *S. enterica* infectivity.[92] Chloramphenicol was one of Nigeria's most common drugs of choice in treating *Salmonella*-related infections. A survey revealed 72.4%–89.2% increased resistance from 1997 to 2007, thus limiting its therapeutic value.[117,118] In Iraq, chloramphenicol-associated genes were highly occurring in *S. enterica* strains isolated from clinical samples.[119,120] In literature, chloramphenicol was the first-line drug used to treat typhoid fever, but its recurrent use limits its therapeutic value due to resistance development.[119,120]

The presence of *inv*A, *sop*B, *spi*4D, and *mgt*C genes in all the tested isolates agreed with the literature's earlier evidence.[13,121,122] *Salmonella* invasion gene (*inv*A) is involved in the invasion of the intestinal epithelium cells and is found in pathogenic *S. enterica.*[123] Therefore, for *Salmonella* infection to occur, invasion of the cells must occur, aided by *inv*A gene.[124–126] The *inv*A gene influences the type of *Salmonella* infection that could result in either systemic or localized.[127] This gene is a transcriptional regulator required to express several genes encoding type III secretion system SPI-1 effector proteins.[57,121,128] The *inv*A gene was previously hypothesized to be widely distributed among the *S. enterica* isolates irrespective of their serovars or source of isolation. Thus, the *inv*A gene is a suitable target for detecting *S. enterica* from different biological specimens, as documented in the literature.[56,60,121,127,129–131]

The inositide phosphate phosphatase (*sop*B) gene is an effector protein that induces macropinocytosis. Gene *sop*B is an actin-binding protein that interacts with the host cell actin cytoskeleton. It is required for efficient bacterial internalization by the host cell.[132] The *sop*B gene in all isolates is instructive because *sop*B has been reportedly involved in micropinocytosis.[133] Macrophages have been considered the main target of *Salmonella* during infection, and these cells are responsible for bacterial dissemination and control.[134–136] In addition to macrophages, other immune system cells are targets of *S. enterica* pathogens, dendritic cells, and neutrophils.

Furthermore, B cells have also been targeted by *S. enterica* through the expression of *sop*B.[137] The *sop*B genes are necessary for intracellular survival in the host, so the presence of *sop*B gene is suggested to contribute to the invasiveness of *S. enterica* pathogens,[138,139] found in all the isolates in this study. The *sop*B gene is also involved in host cell survival by activating the Akt signaling pathway, including activation of the host innate immune system and cell death.[133] The presence of bacterial effector *sop*B in our study supported the earlier assumption that activation of Akt pathway is mediated through the expression of *sop*B.

In literature,[126] *Salmonella*'s SPI-3 island is associated with intra-macrophage invasion, which supports survival when Mg2+, required in the bacteria transported system, is of limited amount. The presence of *mgt*C gene in all the *Salmonella* in this study supported the proposition that *S. enterica* uses the expression of *mgt*C gene to circumvent the lack of Mg2+ in the bacteria. They, therefore, initiate Mg2+ production without depending on the host for Mg2+. Our findings are supported in earlier literature.[13,137,139,140] *Salmonella enterica* contains several transport systems, both inducible and constitutive.[140] These transport systems have functional complementarities to adjust the Mg2+ concentration in different environmental conditions. In addition, these systems are controlled by transcriptional and post-transcriptional regulatory networks to maintain strict control of the Mg2+ balance.[126] Regarding maintaining *Salmonella* viability and development in environments with low Mg2+ levels,

*mgt*C appears to be the most crucial SPI-3 component, as reported in some *S. enterica* isolates[140] and *S. typhi*.[13] Since mgtC is encoded in a region of SPI-3 that is highly conserved, it plays a crucial role in virulence that is not met by any other factor encoded in SPI-3 or anywhere else on the *S. enterica* chromosome.[121,141]

The *ssa*Q gene was detected in 33 (39.8 %) *Salmonella* isolates examined. The importance of this gene is relevant in the surveillance of *S. enterica*, which has been involved in systemic infection in the past. It has been found to produce proteins for the bacteria that bind to and stabilize the larger protein, which is important for the overall efficiency of the secretory system.[142] Essential for virulence in host cells, survival in macrophages, and biofilm development is the *ssa*Q gene, which codes for proteins in the SPI-2 type III secretion system.[143]

The *Salmonella* plasmid virulence (*spv*C) gene was significantly higher in iNTS than in typhoid *Salmonella* isolates. By eliminating their beta-subunits, the *spv*C gene renders inactive the host's dual-phosphorylated mitogen-activated protein kinases. It is also hypothesized to play a role in systemic *S. enterica* infection due to its anti-inflammatory effector effects and attenuation of the intestinal inflammatory response.[144] The *spv*B gene may collaborate with spvC and other *Salmonella* effectors to play a role in pathogenesis by triggering apoptosis in human macrophages. The *spv* genes increase the virulence of non-typhoid *Salmonella* serovars to induce extra-intestinal illness, as shown by experimental models and human epidemiological data.[145] Intestinal infections caused by non-typhoid *Salmonella*, typically present as self-limiting gastroenteritis, can be terminated by spv genes.[129] In mice, a study[145] discovered that the *spv* locus in *Salmonella* serovars is a crucial distinction in the pathogenesis of typhoid fever compared to that of non-typhoid *Salmonella* bacteremia.

Study limitations

As this is a further study on *Salmonella* isolates from an initial isolation process, the sample size was not determined; as such, all the *Salmonella* isolates recovered from 2015 to 2018 were included in this study. Gene sequencing of the antibiotic resistance and the virulence genes of *Salmonella* isolates detected were not done to detect mutations that could adversely affect the activity of the antimicrobial agents and their pathogenicity abilities.

Conclusions

The result of our study illustrates the emergence of multi-drug resistant *S. enterica* from children with bacteremia in north-central Nigeria. The most common antibiotics that *S. enterica* recovered were resistant to were ampicillin and trimethoprim-sulfamethoxazole. Some recovered *S. enterica* demonstrated multi-drug resistance to penicillins, first-generation cephalosporin (cephalothin), phenicol, sulfonamide, tetracycline, aminoglycosides, and fluoroquinolone. None of the *S. enterica* isolates met the criteria required for XDR and PDR designation. The recovered antimicrobial resistance genes (bla_{TEM}, *qnr*A, *flo*R, *tet*A, *tet*B, and *tet*G) were found. The most prevalent gene was bla_{TEM}, while *tet*G was the least prevalent resistance gene. The *inv*A, *sop*B, *mgt*C, and *sip*4D were found in all the recovered *S. enterica* isolates. At the same time, most *S. enterica* also harbored *spv*C and *ssa*Q genes, respectively, with *ljs*GI-1 gene found in only two *S. typhi* isolates. Therefore, this study recommends continuous monitoring of antimicrobial resistance patterns of *S. enterica* from invasive sources in Nigeria and encourages the prudent use of antibiotics and the practices of other infection prevention control measures.

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Author contributions

Leonard Uzairue was involved in conceptualization, investigation, methodology, formal analysis, visualization, and writing; Olufunke Shittu was involved in conceptualization, editing, and supervision; Olufemi Ojo and Tolulope M. Obuotor were involved in editing and supervision; Grace Olanipekun, Theresa Ajose, and Ronke Arogbonlo were involved in project administration and supervision. Nubwa Medugu and Bernard Ebruke were involved in methodology, supervision, and editing; and Stephen Obaro was involved in conceptualization, supervision, and editing.

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Ethical approval and consent to participate

The research got approvals from the Research Ethics Committees of the Federal Medical Centre Keffi (FMC/KF/HREC/052/15), the Nyanya General Hospital, FCT, Abuja (FCTA/HHSS/HMB/NH/GEN/54/II/128), and the University of Abuja Teaching Hospital (FCT/UATH/HREC/PR/61). Written informed consent was obtained from the parent or guardian of the children. The identities of all data/samples used for this study were removed entirely.

Informed consent

Written informed consent was obtained from the parent or guardian of the children.

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Availability of data and materials

All data from this research are available at https://data.mendeley.com/datasets/3njxchzht8/1.

Supplemental material

Supplemental material for this article is available online.

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