

Whole Genome and Phenotypic Characterization of Novel QnrB19-positive *Salmonella* Nigeria serovars from Food Animals in Ilorin, North-central, Nigeria.

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Research article

Keywords: *Salmonella*, Multilocus sequence typing (MLST), QnrB19, Whole-genome sequencing, Antimicrobial resistance, Virulence genes, Plasmids, PMQR, Nigeria

Posted Date: January 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-147029/v1>

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Abstract

Background: Non-typhoidal *Salmonella* are major foodborne pathogens, posing serious challenges to public health and food safety worldwide. Salmonellosis in humans is commonly associated with the consumption of contaminated food, water, and direct contact with infected animals. This study aimed to characterize the distribution, diversity, virulence genotypes and antibiotic resistance of *Salmonella enterica subsp. enterica* serovar Nigeria, isolated from farm animals in north central Nigeria.

Results: We recovered 9 different *S. enterica* ser. Nigeria isolates from our sampling, eight from pig and one from chicken. The antimicrobial susceptibility testing against 15 antimicrobial agents showed variable resistance profiles. Whole genome sequence (WGS) analysis revealed that all 9 isolates contained a single mutation *parC* (T57S) substitution in addition to *qnrB19*, expected to confer decreased susceptibility to ciprofloxacin and *tet(A)* expected to confer resistance to tetracycline. Furthermore, two plasmid targets were also detected in all the strains, Col(pHAD28) and IncQ1. MLST analysis showed that all 9 isolates exhibited only one sequence type, (ST-4911) irrespective of the source of isolation. A SNP-based phylogeny indicates that the 9 isolates are highly related and lack other close relatives in the pathogen detection database.

Forty core (housekeeping) and accessory virulence genes were identified from different virulence loci including *Salmonella* Pathogenicity Islands, virulence associated plasmids (pSV), chromosomes and fimbriae.

Conclusion: This study provided valuable information on the resistance determinants, virulence genes, phenotypic resistance profiles, plasmids and multilocus sequence typing (MLST) of *Salmonella* Nigeria from food animals by WGS. Highlighting the significance of poultry and pig to the spread and emergence of *Salmonella* Nigeria in this region of Nigeria, therefore, there is the need for consumer's education and enlightenments on the importance of proper handling and preparation of food, this will reduce the potential risk of transmission of this pathogen.

Background

Non-typhoidal *Salmonella* is among the most important foodborne pathogens and it continues to pose a serious challenge to public health and food safety globally [1]. Salmonellosis in human are commonly associated with the consumption of contaminated foods, water, and direct contact with infected animals have also been implicated [2]. Gastroenteritis due to non-typhoidal *Salmonella* is usually a self-limiting illness and is characterized by diarrhea, fever, vomiting and abdominal cramps. Usually, children, immuno-compromised and elderly individuals are more likely to develop severe disease with a higher risk of secondary complications.

Over 2,600 *Salmonella* serovars have been reported, but only a few are incriminated in most cases of human salmonellosis worldwide these includes *Salmonella* serovars *S. Enteritidis*, *S. Typhimurium*, *S. Infantis* and *S. Heidelberg* [3], these serovars are commonly reported in poultry and swine farms. Eggs and poultry products have been incriminated as the main vehicles for the transmission of human salmonellosis that is responsible for the majority of foodborne outbreaks [4]. Because *Salmonella enterica* is widely distributed in the environment and global food chain production, in addition to having a large public health impact it also has a huge economic implication estimated at \$11.6 billion [5].

Worldwide, *Salmonella* is a major cause of hospitalizations and mortality among those attributed to foodborne diseases [6] causing an estimated 93.8 million cases and 155,000 deaths per year [2]. Moreover, in Sub-Saharan Africa, invasive nontyphoidal *Salmonella* (INTS) has emerged as a major cause of bloodstream infection in adults and children, with an estimated annual incidence of 175–388 cases per 100,000 children and 2000–7500 cases per 100,000 HIV-infected adults [7]. Over the last decade, the emergence of some serotypes in poultry production has been observed. *Salmonella enterica* serotype Nigeria has been associated with poultry and pigs [8, 9], but recently it has been more frequently observed in pig farms. It was also recently encountered in a human [10] in the south west of Nigeria.

Antimicrobial resistance (AMR) is an increasing problem worldwide, and *Salmonella* spp. resistance to quinolone was classified by World Health Organization (WHO) in the high priority list. AMR in foodborne pathogens is a significant threat to public health, this is particularly true with nontyphoidal *Salmonella*, acclaimed to be the most common bacterial foodborne pathogen in the United States [11]. The Centers for Disease Control and Prevention (CDC) regard *Salmonella* resistance to fluoroquinolones to be a serious threat to public health [12], and the Food and Drug Administration (FDA) identified fluoroquinolones as critically important drugs for human health [13]. Therefore, findings of decreased susceptibility to fluoroquinolones or identification of new genetic determinants conferring fluoroquinolone resistance in *Salmonella* is a public health concern. The emergence of antimicrobial resistance in microorganisms naturally occurs; nevertheless, the increase in the utilization of antimicrobials promotes the natural selection of resistant bacteria [14]. Generally, bacterial virulence factors have a crucial role for systemic infections, the pathogenicity of *Salmonella* serotypes is dependent upon the virulence potential of the microorganism and the host susceptibility to the pathogen. Bacterial virulence factors are necessary for adhesion, invasion and replication inside host cells. Genes such as *invA* and *hilA*, found in SPI, allow *Salmonella* to invade epithelial cells [15, 16]. Besides, *Salmonella* outer proteins (sops) (SPI effector protein) encoded by *sop* gene have relevance to *Salmonella* virulence [17]. Meanwhile, the plasmid encoded fimbriae (*pefA*) gene contributes to the adhesion of *Salmonella* to epithelial cells. Other chromosomal gene like *stn*, that code for enterotoxin production has been shown to be a causative agent of diarrhea. In addition, virulence plasmids carrying virulence genes such as the *spv* operon (*Salmonella* plasmid virulence) contribute to the colonization of deeper tissues among other functions. These characteristics are encoded by genes present on a wide range of genetic elements, including the bacterial chromosome, plasmids, prophages and *Salmonella* Pathogenicity Islands (SPIs). SPI-2, which encodes T3SS-2 that facilitates intracellular survival and replication [18]. Other SPIs are serotype specific and increase the virulence potential of the pathogen, occasionally plasmids are found in *Salmonella* serotypes associated with infections of humans and animals, including the *Salmonella* virulence plasmid.

To provide a deeper understanding of *S. Nigeria* in this region we report the genome sequence of *S. Nigeria*, obtained using MiSeq Illumina instrument with the 500-cycle MiSeq reagent V2 kit (2 × 250 bp). This study aimed to provide baseline information on the distribution, molecular characterization, virulence

genotypes, SNP-based phylogenetics, genotypic and phenotypic antibiotic resistance profiles of *Salmonella* serovar Nigeria isolated from food and food animals.

Materials And Methods

Study area

Samples were collected from 14 farms (pig, n = 9; poultry, n = 5) located at Egbejila, Eyenkorin, and Lasaju communities located near Ilorin metropolis, Kwara State, Nigeria. The geographic position of the farms lies on latitude as shown in Fig. 1. The State is located at an elevation of 305 meters above sea level with population of 2,591,555. Its coordinates are latitudes (8° 30'N) and longitudes (5° 00'E). The state shares a common internal boundary with Niger state in the North, Kogi state in the East, Oyo, Ekiti and Osun states in the South and an international boundary with the Republic of Benin in the West. The state has an annual rainfall range of 1,000 mm to 1,500 mm. The rainy season begins at the end of March and lasts until early September, while the dry season begins in early October and ends in early March. Temperature is uniformly high and ranges between 25 °C and 30 °C in the wet season throughout the season except in July – August when the clouding of the sky prevents direct insolation while in the dry season it ranges between 33 °C to 34 °C.

Sample collection

A cross sectional study of selected pig farms and poultry slaughter houses was conducted. A total of 1,500 samples comprising of pig fecal samples (n = 600) and poultry samples (n = 900) were collected from March, 2014 to September, 2016 at Eyenkorin community and Ilorin town, both in Ilorin metropolis, North-central, Nigeria with the farmer's consent. Twenty-five grams of freshly voided pig fecal sample and tissue/organ samples from poultry were collected from March, 2014 to September, 2016 at Eyenkorin community and Ilorin metropolis, Ilorin, North-central, Nigeria respectively. Fecal samples were obtained from the rectum and pen floor of the pig, tissues/organs were obtained directly after post mortem of dead birds on the farm. Samples were collected directly into sterile buffered peptone water (Oxoid, Basingstroke, Hampshire, England) in sterile universal bottles and transported on ice packs to the veterinary microbiology laboratory, University of Ilorin, Nigeria for analysis.

Isolation and Identification of *Salmonella*

The sample in buffered peptone water (10 gram of sample to 90 milliliter of broth) was incubated at 37 °C for 24 hours, thereafter, one milliliter was inoculated into 9.0 ml of Selenite-F broth (fecal sample) (Fluka Biochemika, Steinheim, Germany), Rappaport-Vassiliadis (for tissues/organs) (Oxoid, Basingstroke, Hants, UK) and incubated for 24 hours at 37° C. The selective broth was streaked on *Salmonella Shigella* agar (Rapid Labs, Colchester, Essex, UK) and incubated for 24 hours at 37° C. Colonies appearing colourless with black centre were sub-cultured on Xylose Lysine Deoxycholate (XLD) agar (Oxoid, Basingstroke, Hants, UK), incubated aerobically at 37° C for 24 hours. Presumptive *Salmonella* isolates on XLD (that appeared pink/red with dark center) were confirmed by standard biochemical tests and stored on nutrient agar (Oxoid, Basingstroke, Hants, UK) slants for serotyping at the WHO National *Salmonella* and *Shigella* Center, Bangkok, Thailand according to the Kauffmann-White Scheme.

Antimicrobial susceptibility testing

Isolates positive for *Salmonella* were subjected to antimicrobial susceptibility tests with antimicrobials disks using disk diffusion method on Muller–Hinton agar (Oxoid, Basingstroke, Hants, UK) plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. *Escherichia coli* ATCC 25922 was utilized as quality control strain. The following antimicrobial disks (Himedia®, Mumbai, India) with the corresponding concentration were used; ampicillin, AMP (10 µg), cloxacillin, OB (5 µg), cefotaxime, CTX (30 µg), ceftazidime, CAZ (30 µg), ceftriaxone, CTR (30 µg), chloramphenicol, C (30 µg), ciprofloxacin, CIP (5 µg), gentamicin, GEN (10 µg), nalidixic acid, NA (30 µg), streptomycin, S (10 µg), neomycin, N (10 µg), Sulpha/Trimethoprim COT (25 µg), tetracycline, TET (30 µg), and trimethoprim, TR (5 µg). Diameters of zones inhibition were measured with Himedia® Antibiotic Zone scale (Himedia®, Mumbai, India) and interpreted according to the CLSI guidelines [19].

Whole genome sequencing

Serotyped isolates were transported on nutrient agar slant to U.S. Food and Drug Administration, College Park, Maryland, USA for WGS analysis. Bacterial DNA was extracted from all the overnight cultures by using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA.) according to the manufacturer's instructions. Sequencing libraries were constructed with 0.2 ng/µl of prepared DNA using the Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA).

Sequencing was carried out by using the MiSeq Illumina instrument with the 500-cycle MiSeq reagent V2 kit (2 × 250 bp) in accordance with the manufacturer's guidelines. The fastq files were uploaded to NCBI's SRA database for inclusion in the GenomeTrakr's [20] open surveillance of foodborne pathogens. The GenBank accession number and other genomic statistic of the study are as shown in Table 1.

Table 1
Genomic statistic of *Salmonella* Nigeria isolated from pigs and poultry in Ilorin, North central Nigeria.

Sample ID	GenBank accession No.	Biosample accession No.	Genome					SNP	No of coding genes	No of CDS	NCBI Pa Detectio Assemb
			Contig N50	GC contents %	No. of Contigs	Coverage	Total sequence length (bp)				
CFSAN083299	GCA_008157485.1	SAMN12601395	223,140	53.2	53	123x	4,607,620	0	4,303	4,506	PDT000
CFSAN083286	GCA_006292415.1	SAMN11897689	173,340	53.4	64	55x	4,613,916	2	4,313	4,420	PDT000
CFSAN083315	GCA_006396655.1	SAMN11898373	207,666	52.5	65	162x	4,604,840	0	4,314	4,419	PDT000
CFSAN083320	GCA_006396575.1	SAMN11897709	174,533	52.4	65	88x	4,611,538	0	4,315	4,415	PDT000
CFSAN083321	GCA_006146085.1	SAMN11897888	172,677	52.4	70	59x	4,607,335	0	4,317	4,453	PDT000
CFSAN083314	GCA_006396615.1	SAMN11897866	172,455	52.6	82	176x	4,625,918	0	4,344	4,453	PDT000
CFSAN083289	GCA_006213365.1	SAMN10505104	178,056	53	57	116x	4,612,844	2	4,381	4,516	PDT000
CFSAN083317	GCA_006080435.1	SAMN11896190	176,123	52.2	76	168x	4,604,193	0	4,316	4,421	PDT000
CFSAN083295	GCA_008157565.1	SAMN12601407	115,483	52.3	92	86x	4,598,172	0	4,326	4,429	PDT000

Whole genome sequence analysis

Raw data were downloaded locally, assembled by using SPAdes v3.8 [21], and annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [22]. *In silico* serotyping was performed with SeqSero v. 1.0 [23]. Resistance determinants in the ResFinder and PointFinder databases were identified in assemblies using starAMR v. 0.4.0. Plasmid genes were identified using Abricate v. 0.8.10 (<https://github.com/tseemann/abricate>) and a modified version of the PlasmidFinder database (<https://github.com/StaPH-B/resistanceDetectionCDC>). Predicted resistance phenotypes were assigned using the determinants detected and the ResFinder and PointFinder drug keys developed by the Centers for Disease Control and Prevention (<https://github.com/StaPH-B/resistanceDetectionCDC>). The virulence genes present in the genome were identified using abricate v. 0.8.10 and the virulence factors database (VFDB).

Multilocus sequence typing (MLST) was carried out using WGS data using the sequence of seven housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. Genome assemblies were uploaded to the Centre for Genomic Epidemiology MLST 2.0 tool (<https://cge.cbs.dtu.dk/services/MLST/>) to assign sequence types (STs) to each study isolate based on the set of alleles derived from the aforesaid seven loci. To further investigate the genetic relationship among isolates in this study SNP phylogenies available at the NCBI pathogens page (<https://www.ncbi.nlm.nih.gov/pathogens/>) were examined.

Results

We recovered nine *Salmonella* Nigeria isolates, eight from pig fecal samples and one from chicken liver (Table 2).

Table 2
WGS profiles of resistant determinants, resistance genes, plasmids and MLST profiles of *Salmonella* Nigeria.

Sample ID.	Sample source	Serotypes	Year	Resistant Determinants			Plasmids	Anticipated Resistance	MLST profiles
				Mutation	PMQR	Resistance genes			
CFSAN083299	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083286	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083315	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083320	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1, Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083321	Chicken	Nigeria	2016	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083314	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083289	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083317	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083295	Pig	Nigeria	2014	parC(T57S)	qnrB19	tet(A)	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911

The genotypic resistance profiles extracted from the WGS analysis showed all the nine *Salmonella* Nigeria isolates possessing three similar resistance determinants (Table 2). A previously described *parC* (T57S) substitution was identified in all the isolates. Some *parC* mutations are associated with reduced susceptibility to fluoroquinolone antibiotics such as ciprofloxacin, however the *parC* T57S mutation is not known to confer high-level resistance alone [24, 25]. In addition to the *parC* mutation a plasmid mediated quinolone resistance (PMQR) gene - *qnrB19*, and a tetracycline resistance gene (*tetA*) were identified. Two plasmid replicon targets, Col(pHAD28) and IncQ1, were also detected in all isolates (Table 2).

The antimicrobial susceptibility results showed variable rates of resistance patterns against 15 antimicrobial agents. All isolates except showed resistance to tetracycline (n = 8; 89%), two isolates showed resistance to streptomycin, and one each of the isolate exhibited resistance to ampicillin, cloxacillin, gentamicin, and sulfa/trimethoprim. Seven isolates showed intermediate resistance to ciprofloxacin (n = 7; 78%), two isolates each showed intermediate resistance to ceftriaxone, nalidixic acid, trimethoprim. All the isolates were susceptible to cefotaxime (n = 9; 100%), eight isolates each were susceptible to ceftaxitin, ceftazidime, neomycin, and chloramphenicol (n = 8; 89% each) while seven each were susceptible to ampicillin, cloxacillin, ceftriaxone, gentamicin, streptomycin, nalidixic acid, trimethoprim, and sulfa/trimethoprim (n = 7; 78% each), two isolates each were susceptible to ciprofloxacin antimicrobial (n = 2; 22%) Table 3.

Table 3
Antimicrobial resistance profiles of *Salmonella* Nigeria isolated from pigs and poultry farms, Ilorin, Nigeria.

Class of antimicrobials	Antimicrobial agent	Total number of <i>Salmonella</i> Nigeria isolates (n = 9)		
		No. of resistant isolates (%)	No. of intermediate isolates (%)	No. of susceptible isolates (%)
Penicillin (P)	Ampicillin (AMP)	1 (11)	1 (11)	7 (78)
	Cloxacillin (OB)	1 (11)	1 (0)	7 (78)
Cephalosporins (C)				
	Cefotaxime (CTX)	0 (0)	0 (0)	9 (100)
	Ceftaxitin (CX)	0 (0)	1 (11)	8 (89)
	Ceftazidime (CAZ)	0 (0)	1 (11)	8 (89)
	Ceftriaxone (CTR)	0 (0)	2 (22)	7 (78)
Aminoglycosides (A)				
	Gentamicin (GEN)	1 (11)	1 (11)	7 (78)
	Streptomycin (S)	2 (11)	0 (0)	7 (78)
	Neomycin (N)	0 (0)	1 (11)	8 (89)
Phenicol (PH)				
	Chloramphenicol (C)	0 (0)	1 (11)	8 (89)
Fluoroquinolone (F)				
	Ciprofloxacin (CIP)	0 (0)	7 (78)	2 (22)
Tetracyclines (T)				
	Tetracycline (TET)	8 (89)	1 (11)	0 (0)
Quinolones (Q)				
	Nalidixic acid (NA)	0 (0)	2 (22)	7 (78)
Sulfonamides (S)				
	Trimethoprim (TR)	0 (0)	2 (22)	7 (78)
	Sulfa/Trimethoprim (COT)	1 (11)	1 (11)	7 (78)

Genes from five *Salmonella* Pathogenic Islands (SPIs) comprising of SPI-1, 2, 3, 5, 11, virulence associated plasmids (pSV), chromosomal and fimbriae encoded virulence genes were identified for core (housekeeping) and accessory virulence genes. In general, all the isolates exhibited 75% (9/12) of the genes investigated in SPI-1, 81.8% (9/11) in SPI-2, 66.7% (2/3) in SPI-3, 75% (3/4) in SPI-5, 50% (1/2) in SPI-11, 0% (0/4) from pSV loci, 100% each for chromosomal, and fimbriae encoded virulence genes. These inferences are indicative of the ability of the isolates to be virulent and highly pathogenic (Table 4).

Table 4
Virulence genes profiles of *Salmonella* Nigeria from pigs and poultry in Ilorin, Nigeria.

Virulence loci	Genes	<i>Salmonella</i> serovars identification number						
		CFSAN083299	CFSAN083286	CFSAN083315	CFSAN083320	CFSAN083321	CFSAN083314	CFSAN083289
SPI-1	<i>invA</i>	+	+	+	+	+	+	+
	<i>sipA,B,C</i>	+	+	+	+	+	+	+
	<i>hilA,hilC</i>	-	-	-	-	-	-	-
	<i>sopA</i>	+	+	+	+	+	+	+
	<i>orgA</i>	+	+	+	+	+	+	+
	<i>prgH</i>	+	+	+	+	+	+	+
	<i>spaN</i>	-	-	-	-	-	-	-
	<i>sptP</i>	+	+	+	+	+	+	+
	<i>avrA</i>	+	+	+	+	+	+	+
SPI-2	<i>ssaR</i>	+	+	+	+	+	+	+
	<i>spiA</i>	-	-	-	-	-	-	-
	<i>sseB,C,E,F,G</i>	+	+	+	+	+	+	+
	<i>ttrC</i>	-	-	-	-	-	-	-
	<i>ssaB/spiC</i>	+	+	+	+	+	+	+
	<i>ssaQ</i>	+	+	+	+	+	+	+
SPI-3	<i>misL</i>	+	+	+	+	+	+	+
	<i>mgtCB</i>	+	+	+	+	+	+	+
	<i>marT</i>	-	-	-	-	-	-	-
SPI-5	<i>pipB</i>	+	+	+	+	+	+	+
	<i>sopB</i>	+	+	+	+	+	+	+
	<i>csgd</i>	+	+	+	+	+	+	+
	<i>pipA</i>	-	-	-	-	-	-	-
SPI-11	<i>cdtB</i>	+	+	+	+	+	+	+
	<i>pagN</i>	-	-	-	-	-	-	-
pSV	<i>rck</i>	-	-	-	-	-	-	-
	<i>spvB</i>	-	-	-	-	-	-	-
	<i>spvC</i>	-	-	-	-	-	-	-
	<i>pefA</i>	-	-	-	-	-	-	-
Chromosomal	<i>sifA,B</i>	+	+	+	+	+	+	+
	<i>sopE2</i>	+	+	+	+	+	+	+
	<i>sseJ</i>	+	+	+	+	+	+	+
Fimbriae	<i>steA</i>	+	+	+	+	+	+	

The sequence types (STs) extracted from the WGS analysis showed that all the isolates belonged to ST-4911 irrespective of the source of isolation. A SNP-based phylogeny generated as part of NCBI's pathogen detection pipeline (<https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/Salmonella/PDG000000002.1911/PDS000046205.2>) indicates that all the isolates sequenced as part of the present study are highly related and lack other close relatives in the pathogen detection database. The NCBI SNP phylogeny does not contain additional isolates from outside of this study and isolates displayed an average of 1 pairwise SNP difference between isolates, with a range of 0–4 SNPs.

Discussion

To the best of our knowledge, this is the first report of whole genomic or detailed characterization of *Salmonella* Nigeria serovars from food animals. In this study, we reported *Salmonella* Nigeria serovars with a prevalence rate of 0.7%, from poultry and pig farms, this is lower than a previous reports on *Salmonella*

in pig and poultry [26] with 2.2% in Shaoyang, China and Fashae and Hendriksen (2013) with 2.6% prevalence rate in Ibadan, Nigeria. The study highlights the prevalence of this emerging serotype on farms in the South-west and North- central region of Nigeria.

Detection of *Salmonella* Nigeria in this study indicates *Salmonella* contamination of poultry and pig farms investigated, this is of veterinary and public health significance because this predisposes the community to public health challenges. Contamination of poultry and pig farms with *Salmonella* may be attributed to low level implementation of hygiene and biosecurity measures as previously reported by Fagbamila et al. [27]. The isolation of the same serovars from pigs and chickens corroborated the important roles played by environment in the transmission of *Salmonella* because some of the pig farms are extensively managed, pigs roam about to scavenge in poultry farm/environment refuse dumps, furthermore rodents and wildlife animals can move from one farm to the other in search of food resulting in cross contamination as earlier reported [28, 29].

This study is similar and of significance when compared to the earlier work by Fashae et al. [9], with report of 3.5% prevalence of *Salmonella* Nigeria serovars and Fagbamila et al. [8] with a prevalence of 0.3% from pooled pig feces from selected pig farms in Ibadan, southwest Nigeria, and commercial poultry farms respectively. Interestingly, Fashae et al. [10] reported the same serovar from diarrhoeic human in Ibadan, South-west, Nigeria. These reports highlight the ubiquitous nature of *Salmonella* in food animals, and the possibility of cross transmission to humans in these regions.

The resistance observed might be related to the indiscriminate application of antimicrobials for therapeutic or prophylactic purposes (tetracycline), it could also be due to their inclusion in feed as a growth promoter or additives especially in poultry and animal husbandry [30], this is a common practice in developing country like Nigeria, this finding is highly disturbing from a public health perspective as many of these traditional (old generation) antibiotics are still widely prescribed in human medicine due to their low cost and widespread availability [31] while the observation of resistance to some of the newer generations of antibiotics are a cause for concerns.

Resistance to sulfonamides (sulfa/trimethoprim), streptomycin, and tetracycline corroborated similar results by Fashae and Hendriksen [9] on *Salmonella* Nigeria serovars in Ibadan, Nigeria, these antimicrobials are widely used in the treatment and prevention of bacteria diseases by farmers without prescription by veterinarian, hence, the observed in some of the isolates. Other pertinent observations on the outcome of this study is the absence of resistance to chloramphenicol and neomycin, this may be due to the official ban on the use of chloramphenicol in animal, this invariably influenced the use of a "triple" antibiotic formulation/combination (neomycin, chloramphenicol and oxytetracycline) by the farmers, this eventually may be the reason for the drastic reduction in resistance to chloramphenicol and neomycin. It is noteworthy to observe that none of the isolates showed multidrug resistant (MDR) profiles.

In this study the plasmid mediated quinolone resistance gene *qnrB19*, associated with decreased susceptibility to fluoroquinolones, was detected in all the strains. In recent times there have been several reports of foodborne *Salmonella* enterica harboring quinolone resistance genes in Nigeria [9, 32]. These reports showed that public health risk of plasmid-borne resistant foodborne pathogens has emerged globally, this equally confirmed that PMQR genes were located in conjugative plasmids, suggesting that PMQR genes in foodborne isolates may play a role in the spread of fluoroquinolone resistance through the food chain. Therefore, continuous monitoring is necessary for all resistance determinants in relation to the severity of the risk in foods.

Similarly, we identified a single mutation in *parC* (T57S), similar to report by Kim et al. [33]. This *parC*(T57S) mutation usually cannot independently confer quinolone resistance, this is corroborated by previous study [33, 34]. Interestingly, we detected two plasmid targets, IncQ1, a plasmid associated with resistance to tetracycline encoded by *tetA*, and Col(pHAD28), which has been observed to harbor quinolone resistance genes. IncQ1 plasmid is a group of non-conjugative but mobile plasmids that are stably maintained and found in a wide range of bacteria. They have been incriminated in the spread of antimicrobial resistance genes and emergence of multidrug resistant bacteria. They have been involved not only in conferring resistance to tetracyclines but to other antimicrobials; sulphamethoxazole, streptomycin and tetracycline (pNUC) while the IncQ1 plasmid found in the *Escherichia coli* strain harbored an additional *dfrA14* gene that confers resistance to trimethoprim inserted within the *strA* gene [35].

Generally, our results demonstrated that the PMQR gene *qnrB19* is common in *Salmonella enterica* Nigeria isolated from food animals in Nigeria. The occurrence of these antimicrobial resistance elements in *Salmonella* Nigeria is of public health and food safety concern, and it indicates the need for increased surveillance for the presence of these plasmids in *Salmonella* strains and to assess their actual impact in the rise and spread of quinolone resistance.

Genes encoded by SPI-1 region are essential for the invasion of the intestinal epithelium, these genes includes, *avrA*, *invA*, *sipA*, *sipB*, *sipC*, *sopA*, *orgA*, *prgH* and *sptP*, all are involved in host cell invasion and enteropathy, they are also responsible for the ability of the pathogen to invade the intestinal epithelial cells, all of these are harbored by *Salmonella enterica* serovar Nigeria, only two genes, *spaN*, and *hilA* were not identified in all the isolates. SPI-2 encoded genes detected includes *ssaB/spiC*, which assists in the survival of the pathogen within the *Salmonella*-containing vacuoles, others includes *ssaR*, *sseB*, *sseC*, *sseF*, and *sseG* genes which are responsible for the intracellular survival and replication of *Salmonella* in the host cell, thereby enhancing the pathogenicity of *Salmonella*, the *ttrC* and *spiA* genes are not detected in all the isolates. The three genes investigated from SPI-3 region are *misL*, which enhances the long term persistence of the pathogen in host, *marT*, *mgtB*, and *mgtC* both enhances the survival of the pathogen within the macrophages, and also responsible for Magnesium transport system, this ultimately favors the intracellular survival of the pathogen in the host, only *marT* gene was not identified among the isolates. Encoded in SPI-5 are *pipB*, *sopB*, and *csgD* genes, these genes are utilized by the pathogen for intestinal epithelial invasion and colonization leading to enteric salmonellosis. *sopB* was identified, this prevents apoptosis of intestinal epithelial cells, and also play an important role in the induction of fluid secretion by enterocytes, as well as in polymorphonuclear leucocytes induction in the intestine. In addition, *sopB* also presents inositol phosphate phosphatase activity, which is directly related to the induction of diarrhea, while *csgD* plays an important role in biofilm formation leading to enhanced capability of the pathogen to respond to starvation, *pipA* was not detected in all the isolates. From SPI-11 *cdtB* was the only gene identified in all the isolates corroborating the work of Suez et al., [36] and Pornsukarom et al. [37]. The gene *cdtB* is responsible for the release of the cytolethal-distending toxin, thereby assisting intra-macrophage survival of the pathogen. The gene equally causes DNA destruction in intoxicated cells, this induces cell cycle arrest, chromatin fragmentation, cell distention and nucleus enlargement. The *cdtB* toxin may contribute to the pathogenicity in human and animal. The presence of *cdtB* has

been reported to be associated with higher rates of invasive disease [38], *pagN* genes was not detected in all the isolates under study. All of the pSV associated genes, *rck*, *spvB*, *spvC*, and *pefA* were not harbored by all the isolates investigated (Table 4). Some effector proteins are encoded on the outside the SPIs and translocated to the host cell by T3SS, these protein/factors are present in other parts of the chromosomes, these includes *sifA*, *sifB*, *sopE2*, and *sseJ*, all of these were identified in this study. *SifA* effector proteins function to alter host cell physiology and promote bacterial survival in host tissues. This protein is required for endosomal tubulation and formation of *Salmonella*-induced filaments (Sifs), Sif formation is associated with intracellular bacterial replication, *sopE2* this is involved in cytoskeleton rearrangements and stimulates membrane ruffling thereby promoting bacterial entry into non-phagocytic cells. *SseJ* effector proteins function to alter host cell physiology and promote bacterial survival in host tissues. This protein is required for endosomal tubulation and negatively regulates the formation of *Salmonella*-induced filaments (Sifs) in epithelial cells. *SteA* is a fimbriae associated genes, was detected in all the isolates it function as an effector proteins which function to alter host cell physiology and promote bacterial survival in host tissues.

The outcome of this study with regards to virulence genes is in agreement with studies of [39] that reported 100% detection of *invA* genes in Egypt and Rahman [40] reported 100% detection of *sopB* in India, but a lower rate of 41.18% was reported by (Ammar et al. [39] also in India. Han et al. [41] reported 100% of the isolates to be positive for *sopB*, *sopE*, and *invA* genes, similar to our study, but in contrast to our work he detected 98% of *hilA*, *prgH*, *avrA*, and reported *spvC* (78.6%), *pefA* (57.1%), which were absent in our study. However, in Italy, Capuano et al. [42] reported rates of lower *sopE* (85.7%) as compared to results of the present study. In tandem with our study, Campioni et al. [43] detected *invA*, *sipA*, *sopB*, *ssaR*, and *sifA*, genes in all of the strains (100%). Similar to our work, Suez et al., [36] and [37], detected *cdtB* genes in their studies in Israel and US respectively. In our study, *sseJ* and *cdtB*, genes were identified this is similar to the work of Pornsukarom et al. [37] and Suez et al. [36]. The *cdtB* toxin (typhoid toxin) may contribute to the pathogenicity in human and animal.

Multilocus sequence types result revealed that a single sequence type, ST-4911 was assigned to all the isolates investigated.

Conclusion

In conclusion, the data presented in this study provided valuable information on the antimicrobial and virulence genes content, the multi-locus sequence types (MLST's), and phylogenetic relationship of a newly emerging *Salmonella* Nigeria serovar from food animals by WGS. This study showed that poultry and pig farms contributed to the spread and emergence of nontyphoidal *Salmonella* Nigeria serovar in Ilorin, North-central Nigeria. We also characterized some important antimicrobial resistance genes by WGS. The absence of multi-drug resistance to majority of the commonly available antimicrobial agents that are commonly used for clinical chemotherapy in human and veterinary practices is a positive development and requires continuous monitoring and public enlightenment to sustain this status to protect public health safety since the strain was initially reported in pig, and it is now reported in poultry and human. Furthermore, identification of variable virulence genes that has the capacity to elicit high pathogenicity on the host is a cause for public health concern. Therefore, systematic surveillance programs of antimicrobials, and legislation on the imported food of animal origin should be considered by the policy makers in Nigeria to curtail the spread of antimicrobial resistance infection to human. Furthermore, the isolation of *Salmonella* from poultry and pig meant for human consumption highlights the need for consumer's education and enlightenments on the importance of proper handling and preparation of food in order to reduce the potential risk of transmission of this pathogen. Our findings provide useful baseline information that will benefit future researchers to use this genomic characterization for further understanding of the mechanism of *Salmonella* serovar Nigeria in induction of pathogenesis of infection in a susceptible host.

Declarations

Acknowledgements

Authors wish to acknowledge the staff of Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, University of Ilorin for their technical supports towards the completion of this research work and Mr. Solomon Oyeniyi, of Department of Geography, University of Ilorin for assistance with the map.

This work was supported by the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration.

Authors' contributions

RIA, CJC, TRE, and HRS analyzed Whole Genome Sequencing data sets; RIA drafted the manuscript; RIA, AOA, and AA were responsible for sampling, isolation and characterization of the various bacterial isolates; RIA, AJA, and AAG conceptualized and designed the study; all authors read and approved the final manuscript.

Funding

This work was not supported by any fund.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files] and where applicable, hyperlinks to publicly archived datasets analyzed or generated during the study are provided.

Ethics approval and consent to participate

The ethical review committee of University of Ilorin, Faculty of Veterinary Medicine, approved this study (FVER/001/2016).

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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Figures

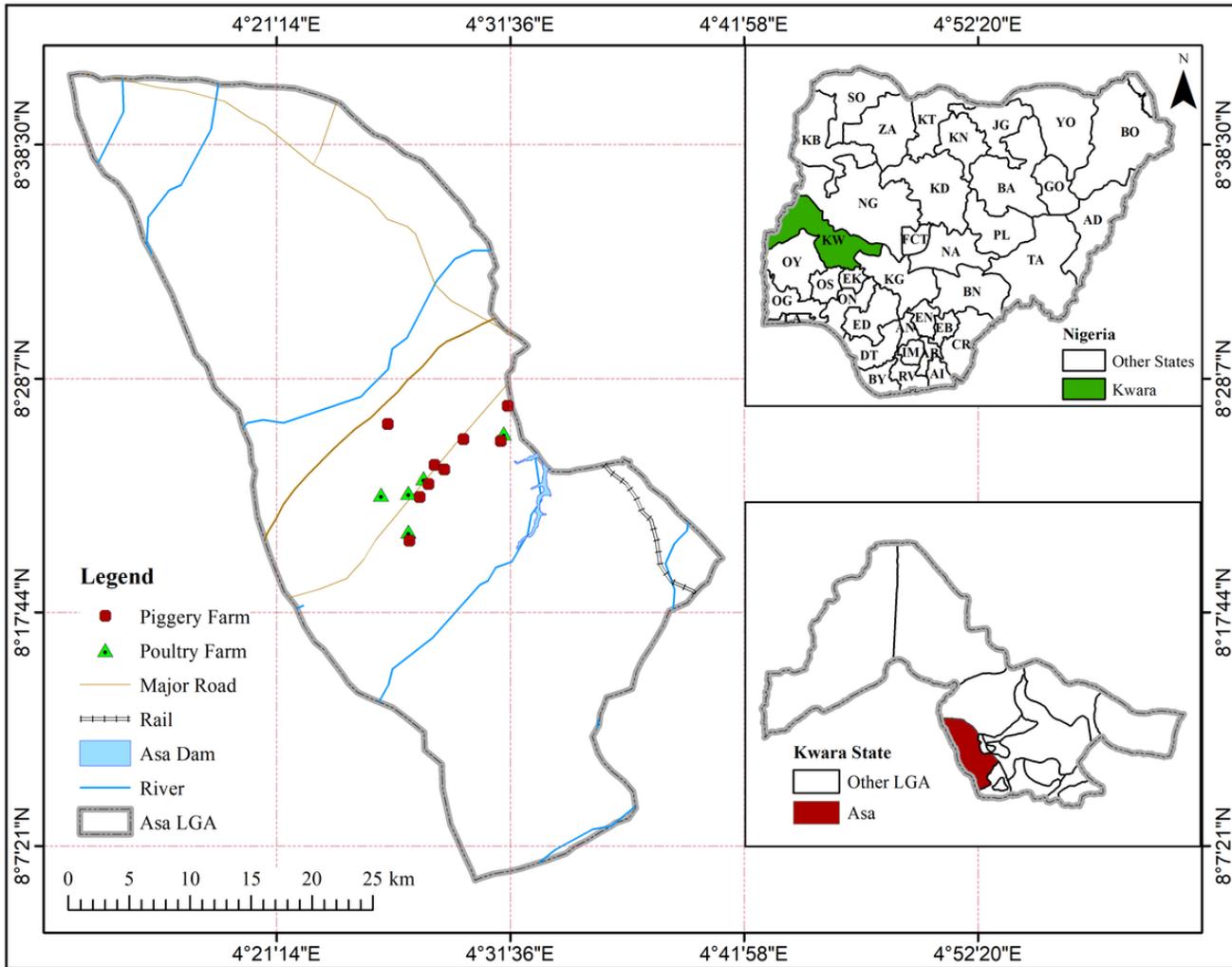


Figure 1

Locations of farms where samples were collected. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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