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Original Article

Antimicrobial and Genomic Characterization of *Salmonella* Nigeria from Pigs and Poultry in Ilorin, North-central, Nigeria

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Abstract

Introduction: Non-typhoidal *Salmonella* are major foodborne pathogens causing serious challenges to public health and food safety worldwide. This study aimed to determine the resistance, virulence genes, sequence type, using multi-locus sequence typing, plasmids and Single Nucleotide Polymorphisms (SNPs) of *Salmonella enterica* subsp. *enterica* serovar Nigeria (*S*. Nigeria) from livestock in Ilorin, North central Nigeria.

Methodology: A total of 1,500 samples from pig (feces; n = 600) and poultry (feces, postmortem samples; n = 900) were collected and analyzed between 2014 to 2017. Presumptive *Salmonella* isolates were characterized by Whole Genome Sequencing (WGS).

Results: We recovered nine *S*. Nigeria serovars. All the isolates harbored a single point mutation *parC*(T57S) in addition to *qnrB19* and the *tetA gene*. Furthermore, two plasmids, Col(pHAD28) and IncQ1 predicted to encode *qnr*B19 and *tetA* genes, respectively, were detected in all the strains. All the isolates belonged to a single sequence type (ST) 4911, the SNP-based phylogeny showed all the isolates to be highly related, in addition two clinical isolates from the United Kingdom (UK) and Canada, collected outside of this study, also fell into this cluster. Twenty virulence genes were identified from *Salmonella* Pathogenicity Islands (SPI), chromosomal and fimbriae loci.

Conclusions: This study highlights the roles of pig and poultry in the emergence and spread of *S*. Nigeria serovar in Nigeria, sub-Sahara Africa. It also highlighted the importance of WGS in clinical and epidemiological surveillance. There is the need for collaborative research studies to investigate the public health importance of *Salmonella enterica* serovar Nigeria.

Key words: *Salmonella enterica* Nigeria; MLST; antimicrobial resistance; virulence genes; plasmids; genomics.

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Introduction

Non-typhoidal *Salmonella* is a major zoonotic pathogen that is responsible for outbreaks and sporadic cases of gastroenteritis in humans globally. Salmonellosis in humans is commonly associated with consumption of contaminated food of animal origin [1,2]. Gastroenteritis due to non-typhoidal *Salmonella* is usually a self-limiting illness and is characterized by diarrhea, fever, vomiting and abdominal cramps. Children, immuno-compromised and elderly individuals may have a higher risk to develop severe disease with a higher risk of secondary complications. Worldwide, Non-typhoidal *Salmonella* (NTS) disease is a major cause of diarrheal disease, and it is estimated to cause 93 million enteric infections and 155,000 diarrheal deaths annually [3]. Moreover, in Sub-Saharan Africa, invasive non-typhoidal *Salmonella* (iNTS) has emerged as a major cause of bloodstream infection in adults and children, leading to an average annual incidence of 370 cases per 100,000 children and 6,000 cases per 100,000 HIV-infected adults [4]. Over 2,750 *Salmonella* serotypes have been reported with some common serovars incriminated in most cases of human salmonellosis worldwide, these includes

Salmonella enterica subsp. *enterica* serovar Enteritidis (*S.* Enteritidis), *S.* Typhimurium, *S.* Infantis and *S.* Heidelberg [5,6], these serovars are commonly reported in poultry and swine farms. Because *Salmonella enterica* is widely distributed in the environment and in the global food chain, there are economic implications estimated at 11.6 billions of dollars [6].

Serotyping is important for *Salmonella* characterization, because the serovar often defines the possible pathogenic potential, host range and disease sequelae [7]. Serotyping therefore forms the basis for national and international *Salmonella* surveillance networks [8,9]. Whole genome sequencing (WGS) is a molecular method for characterizing organisms and has improved our capability to discriminate between closely related strains compared to previously deployed methods. More importantly, it has proven to be a rapid, cost-effective method for infectious disease surveillance [10].

Until now, serological method, based on the reactions of rabbit antisera to the lipopolysaccharide, flagellar antigens and the surface antigen Vi was conventionally used for *Salmonella* classification into more than 2,750 serovars by the White Kauffmann Le Minor Scheme [11]. But in recent times, sequence based techniques such as Multi Locus Sequence Typing (MLST) and Whole Genome Sequencing (WGS) has been introduced for serotyping to identify evolutionary and epidemiological relatedness [7,8,12]. With this method, serotypes prediction can be accomplished by utilizing the freely available *in silico* pipelines, such as SeqSero, which utilizes surface antigen-encoding genes for predicting serotypes [5,13]. The SNP analysis pipeline normally groups isolate into clusters of ascending levels of genetic similarity. The clusters can be detected using analysis of WGS data by comparing isolates based on their single nucleotide polymorphism (SNP) 'address' [7]. The application of WGS increases the sensitivity of cluster detection by identifying additional genetically related isolates that are geographically dispersed which might have previously been considered to be sporadic even if temporally linked [10]. In Nigeria, few studies have utilized WGS in *Salmonella* surveillance and outbreak investigation to determine the sequence types (ST), antimicrobial resistance profiles (including resistance genes), virulence genes, and plasmid replicons [6,14].

To understand the basis and methods of antibiotic resistance transfer in bacteria, comprehensive plasmid sequencing from *Salmonella* isolates obtained from different sources is necessary. In recent times, plasmid sequence extracted from WGS data is increasingly being used and to date, different types of bioinformatics tools (e.g., PlasmidFinder, PlasmidSPAdes, Recycler, PLACNET, etc.) have been developed for in silico detection and characterization of plasmids in whole genome sequences [13,15].

Previously in Nigeria, several studies on *Salmonella* with resistance to several antimicrobials have been reported in humans and veterinary medicine [14,16,17], with very little or lack of information on the plasmids encoding these resistance genes.

Antimicrobials are important for the treatment of microbial infections in both humans and animals; it is therefore pertinent to preserve their effectiveness. The emergence of antimicrobial resistance in microorganisms naturally occurs, nevertheless, the increase in the utilization of antimicrobials as growth promoters in farm animals can support the natural selection of resistant bacteria.

Antimicrobial resistance that emanated from animal husbandry is now well established and affects zoonotic pathogens such as *Salmonella serovars* [18]. More importantly, the use of antimicrobials indiscriminately without prescription can lead to the emergence and dissemination of antimicrobial resistance among the bacterial populations, this has resulted in a major public health concern [19]. The spread of antimicrobial resistance is enhanced by the presence of resistance genes on mobile genetic elements such as plasmids, integrons and transposons, that are transferable within or between different species of bacteria by horizontal gene transfer mechanisms [20].

Over the years, there have been increasing reports on the occurrence of plasmid mediated quinolone resistance (PMQR) harbored by several *qnr* genes in Nigeria [21–23] which protect bacterial topoisomerases from the actions of quinolones. Though, these plasmids do not induce high level resistance, their presence only enhances mutation in the quinolone resistance-determining regions (QRDRs) [24]. Therefore, detection of decreased susceptibility to fluoroquinolones or identification of new genetic determinants conferring fluoroquinolone resistance in *Salmonella* signifies a threat to public health. Generally, bacterial virulence factors are necessary for adhesion, invasion and replication within the host cells. Virulence genes play a crucial role in systemic infections, the pathogenicity of *Salmonella* serotypes is usually dependent upon its virulence potential and the host susceptibility to the pathogen.

In the past, the emergence of some serotypes in poultry production has increased, especially in Nigeria [22]. *Salmonella* Nigeria is a rarely reported serovar

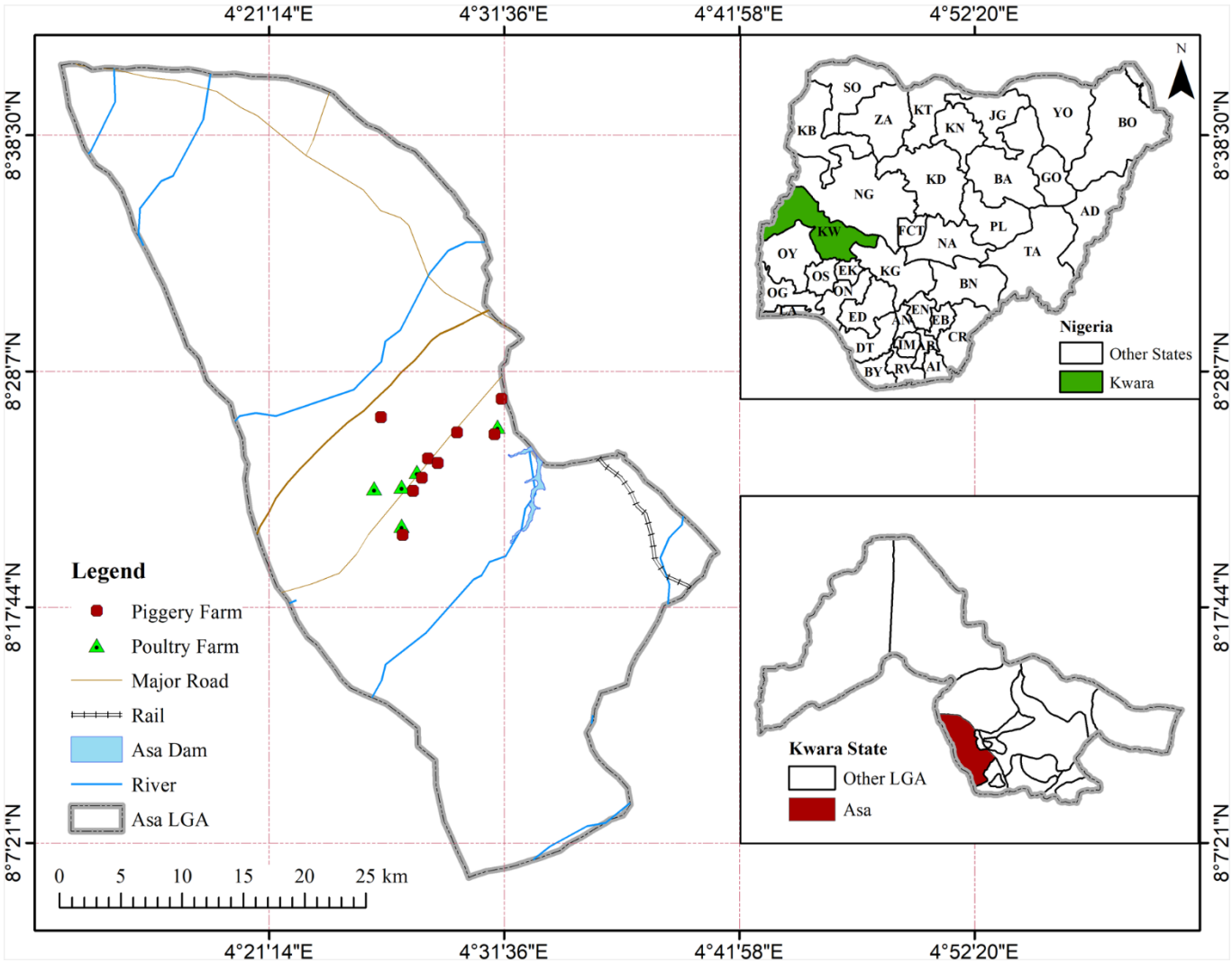
globally, it was first isolated from feces of apparently healthy human and cattle in Ibadan, South west Nigeria in the late 1950's [25]. In recent times, the increase in *S.* Nigeria isolation in Nigeria, especially across the geographical regions is a cause for concern. More importantly, it is being isolated from human and foods of animal origins [14,26,27]. Because it is not a commonly reported serotype, very little is known about its resistance potentials, plasmid profile, its virulence capability(ies), sequence types or its importance and roles as a zoonotic pathogen. In order to evaluate its potential to cause disease in human and animals, the virulence factors database (VFDB) were searched for twenty virulence factors from *Salmonella* pathogenicity islands (SPIs) including SPI-1, SPI-2, SPI-3, SPI-5, SPI-11, chromosomal and fimbriae loci, furthermore, the sequence types, its plasmid/plasmid replicons, and associated antimicrobial resistance capability were investigated using whole genome sequencing (WGS) methods that are just emerging in Nigeria. This study aimed to, for the first time, determine the antimicrobial resistance, plasmids, sequence type and virulence genes of *Salmonella enterica* serovar Nigeria obtained from pig and poultry sources from Ilorin, North central Nigeria using WGS methods.

Methodology

Study area

The study was conducted in three locations; Egbejila, Eyenkorin, and Lasoju town near Ilorin metropolis, Kwara state, North central Nigeria (Figure 1). The state is located on 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,

Figure 1. Locations of farms where samples were collected.



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, rain - season begins in March and ends in September, the dry - season starts in early October and ends in March. Temperature is uniformly high and ranges between 25°C and 30°C in the wet season except during 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 farms was conducted from March 2014 to September 2016. Three types of livestock agricultural practices exist in Nigeria: extensive, semi-intensive, and intensive livestock practices. The extensive system involves rearing of small number of local, and occasionally few exotic breeds of livestock, these are allowed to roam about scavenging for food. The intensive system involves large-scale or commercial production of high performance livestock, very common in urban areas. It is capital intensive and is usually operated by multinationals or wealthy individuals. The semi-intensive system is midway between the intensive and extensive systems [22].

A total of 1,500 samples from apparently healthy pig (feces; n = 600) and poultry (feces, postmortem tissues/organs; n = 900) were collected with farmer's consent. Twenty-five grams of freshly voided fecal and tissue/organ samples were aseptically collected with sterile sample bottle. Fecal samples were obtained from the rectum (pig) and pen floor, cloaca and litter (chicken) or from tissues/organs, directly after post mortem on the farm. Samples were kept on ice and immediately transported to the Department of Veterinary Microbiology laboratory and stored in the refrigerator at 4ºC on the day of collection until samples are analyzed.

Isolation and Identification of Salmonella

25 g of sample was transferred to 225 mL buffered peptone water (Oxoid®, Basingstroke, Hants, UK) and incubated aerobically at 36ºC ± 2ºC for 18-24 hours, thereafter, one milliliter of the overnight broth was dispensed into 9.0 ml of Selenite-F broth (for fecal sample) (Fluka Biochemika®, Steinheim, Germany), Rappaport-Vassiliadis (for tissues/organs) (Oxoid®, Basingstroke, Hants, UK) and incubated aerobically for 18-24 hours at 37ºC ± 2ºC. The broth was streaked on *Salmonella Shigella* agar (Rapid Labs, Colchester, Essex, UK) and incubated aerobically for 18-24 hours at 37ºC ± 2ºC. Colonies appearing colorless with dark center were sub-cultured onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid®, Basingstroke, Hants, UK) and incubated aerobically at 37ºC ± 2ºC for 18-24 hours. Presumptive *Salmonella* isolates on XLD (that appeared pink/red with dark center) were confirmed by biochemical tests: Voges–Proskauer, methyl red, citrate utilization, triple sugar iron agar, urease, oxidase, and hydrogen sulfide and interpreted according to Feltham and Barrow [28] and stored on nutrient agar (Oxoid®, Basingstroke, Hants, UK) slants for serotyping according to White-Kauffmann-Le Minor scheme as previously described [22].

Antimicrobial susceptibility testing

All *Salmonella* positive isolates were selected for antimicrobial susceptibility tests by disk diffusion method on Muller–Hinton agar (Oxoid®, Basingstroke, Hants, UK) plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines with *Escherichia coli* ATCC 25922 as quality control strain. The following antimicrobial disks (Himedia®, Mumbai, India) with the corresponding concentration were used: ampicillin, AMP (10 µg), cefotaxime, CTX (30 µg), cefoxitin, CX (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 (25µg), tetracycline, TET (30 µg), and trimethoprim, TR (5 µg). Diameters of inhibition zones were measured with Himedia® Antibiotic Zone scale (Himedia®, Mumbai, India) and interpreted according to the CLSI guidelines [29].

Whole genome sequencing (WGS)

One hundred and nine presumptive *Salmonella* isolates were shipped on nutrient agar slants to U.S. Food and Drug Administration, College Park, Maryland, USA for whole genome sequencing. Bacterial DNA was obtained from the overnight cultures by utilizing DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA.) in accordance to the manufacturer's instructions. Sequencing libraries were constructed by using 0.2 ng/µl of prepared DNA by utilizing the Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA).

Sequencing was conducted on a 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 National

Table 1. Genomic statistics of *Salmonella enterica* Nigeria serovar 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 Pathogen Detection Assembly
			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	PDT000568622.1
CFSAN083286	GCA_006292415.1	SAMN11897689	173,340	53.4	64	55x	4,613,916	2	4,313	4,420	PDT000510586.1
CFSAN083315	GCA_006396655.1	SAMN11898373	207,666	52.5	65	162x	4,604,840	0	4,314	4,419	PDT000510631.1
CFSAN083320	GCA_006396575.1	SAMN11897709	174,533	52.4	65	88x	4,611,538	0	4,315	4,415	PDT000510587.1
CFSAN083321	GCA_006146085.1	SAMN11897888	172,677	52.4	70	59x	4,607,335	0	4,317	4,453	PDT000510606.1
CFSAN083314	GCA_006396615.1	SAMN11897866	172,455	52.6	82	176x	4,625,918	0	4,344	4,453	PDT000510592.1
CFSAN083289	GCA_006213365.1	SAMN10505104	178,056	53	57	116x	4,612,844	2	4,381	4,516	PDT000412868.1
CFSAN083317	GCA_006080435.1	SAMN11896190	176,123	52.2	76	168x	4,604,193	0	4,316	4,421	PDT000510252.1
CFSAN083295	GCA_008157565.1	SAMN12601407	115,483	52.3	92	86x	4,598,172	0	4,326	4,429	PDT000568629.1

Center for Biotechnology Information's (NCBI) SRA database for inclusion in GenomeTrakr [30] open surveillance of foodborne pathogens. NCBI accessions and other genomic statistic of the study are shown in Table 1.

Whole genome sequence analysis

Raw data were locally downloaded from SRA, assembled using SPAdes v3.8 [31], and annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [32]. *In silico* serotyping was performed with SeqSero v.1.0. [9] and resistance determinants in the ResFinder and PointFinder databases were detected in assemblies utilizing starAMR v. 0.4.0 [33], (https://github.com/phac-nml/staramr). Plasmid genes were analyzed by 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). Selected 20 virulence genes were identified using Abricate v. 0.8.10 and the virulence factors database (VFDB) [34] as shown in Table 2.

Multilocus sequence typing (MLST) was conducted using WGS data on the sequence of seven housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. Genome assemblies were subsequently uploaded to the Centre for Genomic Epidemiology MLST 2.0 tool (https://cge.cbs.dtu.dk/services/MLST/) to allocate sequence types (STs) to each of the study isolate on the basis of set of alleles derived from the seven loci. To

Table 2. Virulence genes, locations and functions.

Virulence factors	Location	Functions	Reference
invA	SPI-1	Encodes a protein in the inner membrane of bacteria, necessary for host epithelial cells invasion	[41]
hilA	SPI-1	Regulates type III secretion apparatus, activating the expression of invasion genes	[42]
avrA	SPI-1	Inhibition of both epithelial cells apoptosis and macrophage pyroptosis	[43]
ssaR	SPI-2	Favor the survival and replication within macrophages and subsequent systemic spread	[44]
spiA	SPI-2	Associated with biofilm formation	[45]
*sse*B	SPI-2	Prevents the phagocyte NADPH oxidase from tracking toward SCVs	[46]
*ttr*C	SPI-3	Catalyzes the reduction of tetrathionate to thiosulfate.	[47]
*mis*L	SPI-3	Associated with cellular invasion and survival within the cell	[48]
*mg*tC	SPI-3	Encodes a high-affinity magnesium transporter that contributes to intracellular survival and survival under low magnesium conditions	[49]
marT	SPI-3	Encodes a transcriptional regulator that induces the expression of *MisL*.	[50]
sopB	SPI-5	Involved in epithelial cell adhesion, phagocytes and non-phagocytes cell-invasion	[44]
pipB	SPI-5	Involves in glycolipid biogenesis	[51]
*csg*D	SPI-5	Encodes for a transcriptional regulator of the LuxR superfamily and controls the two extracellular matrix compounds	[52]
pipA	SPI-5	Host recognition/invasion	[53]
cdtB	SPI-11	Encodes the typhoid toxins	[54]
pagN	SPI-11	Contributes to the adherence to mammalian cells.	[55]
sifA	Chromosome	Regulates kinesin accumulation in the SCV, modulates vesicular trafficking, SCV perinuclear migration, SCV membrane integrity	[56]
sopE2	Chromosome	Involves in Actin cytoskeletal rearrangements, invasion of epithelial cells, induction of pro-inflammatory response	[57]
*sse*J	Chromosome	Promotes SIF formation and maintenance of the integrity of the SCV membrane.	[58]
steA	Fimbriae	Control of *Salmonella*-containing vacuole membrane dynamics	[59]

SCV: *Salmonella*-containing vacuole; SIF: *Salmonella*-induced filament; NADPH: Nicotinamide Adenine Dinucleotide Phosphate.

Table 3. Resistance Determinants, plasmids and MLST profiles of *Salmonella enterica* Nigeria serovar from pigs and poultry in Ilorin, North central Nigeria.

Sample ID.	Sample source	Sample type	Resistance Determinants			Plasmids	Anticipated Resistance	MLST profiles
			Mutation	PMQR	Resistance genes			
CFSAN083299	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083286	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083315	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083320	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1, Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083321	Chicken	Liver	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083314	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083289	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083317	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911
CFSAN083295	Pig	Fecal	*par*C(T57S)	qnrB19	*tet*A	IncQ1,Col(pHAD28)	Ciprofloxacin (I/R), Tetracycline	4911

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

Out of 109 presumptive *Salmonella* isolates from 1,500 samples collected, 91 (6.1 %) were positive for *Salmonella* by SeqSero, while 9 (0.6 %) were confirmed to be *S.* Nigeria with eight from pig and one from chicken (Table 3).

The genomic antimicrobial resistance profiles showed all *Salmonella* isolates to possess three similar resistant determinants. Previously described *parC* (T57S) substitution was identified in all the isolates. In addition to the *parC* mutation, a plasmid mediated quinolone resistance (PMQR) gene - *qnrB19*, and a tetracycline resistance gene *tet(A)* were identified.

Two plasmids, IncQ1 predicted to encode *tetA* gene and Col(pHAD28) which harbored quinolone resistance genes *qnr* B19 were detected (Table 3).

Phenotypic antimicrobial susceptibility tests showed intermediate or low-level resistance against the 14 antimicrobial agents. All the isolates showed phenotypic resistance to tetracycline, and intermediate or low-level resistance to ciprofloxacin (21-30 mm). They were susceptible to all the remaining antimicrobials (Table 4).

Out of the 20 virulence factors investigated, 14 (70%) were detected in all the isolates while six were

Table 4. Antimicrobial resistance profiles of *Salmonella enterica* Nigeria serovar isolated from pigs and chicken in Ilorin North central Nigeria.

Antimicrobial agent	Total number of *Salmonella enterica* Nigeria isolates (n = 9)		
	No. of resistant isolates (%)	No. of intermediate isolates (%)	No. of susceptible isolates (%)
Penicillin (P)			
Ampicillin (AMP)	0 (0)	(0)	9 (100)
Cephalosporins (C)			
Cefotaxime (CTX)	0 (0)	0 (0)	9 (100)
Cefoxitin (CX)	0 (0)	0 (0)	9 (100)
Ceftazidime (CAZ)	0 (0)	0 (0)	9 (100)
Ceftriaxone (CTR)	0 (0)	0 (0)	9 (100)
Aminoglycosides (A)			
Gentamicin (GEN)	0 (0)	0 (0)	9 (100)
Streptomycin (S)	0 (0)	0 (0)	9 (100)
Neomycin (N)	0 (0)	0 (0)	9 (100)
Phenicols (PH)			
Chloramphenicol (C)	0 (0)	0 (0)	9 (100)
Fluoroquinolone (F)			
Ciprofloxacin (CIP)	0 (0)	9(100)	0 (0)
Tetracyclines (T)			
Tetracycline (TET)	9(100)	0 (0)	0 (0)
Quinolones (Q)			
Nalidixic acid (NA)	0 (0)	0 (0)	9 (100)
Sulfonamides (S)			
Sulfa/Trimethoprim (COT)	0 (0)	0 (0)	9 (100)
Folate pathway inhibitor			
Trimethoprim (TR)	0 (0)	0 (0)	9 (100)

Ampicillin: AMP (10 µg); cloxacillin: OB (5 µg); cefotaxime: CTX (30 µg); cefoxitin: CX (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 (25µg); tetracycline: TET (30 µg); and trimethoprim: TR (5 µg); Penicillin (P); Cephalosporins (C); Aminoglycosides (A); Phenicols (PH); Fluoroquinolone (F); Tetracyclines (T); Quinolones (Q); Sulfonamides (S).

Table 5. Virulence gene profiles of *Salmonella enterica* Nigeria serovar from pigs and chicken in Ilorin, North central Nigeria.

Sample ID No.	Virulence loci																			
	SPI - 1				SPI - 2				SPI - 3				SPI - 5		SPI - 11		Chromosomal			Fimbriae
	invA	*sopB*	*hilA*	*avrA*	*ssaR*	*spiA*	*sseB*	*ttrC*	*misL*	*mgtC*	*marT*	*pipB*	*csgD*	*pipA*	*cdtB*	*pagN*	*sifA*	*sopE2*	*sseJ*	*steA*
CFSAN083299	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083286	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083315	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083320	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083321	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083314	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083289	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083317	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+
CFSAN083295	+	+	−	+	+	−	+	−	+	+	−	+	+	−	+	−	+	+	+	+

absent, of significance was the detection of *invA* genes in all the isolates (Table 5).

The MLST results showed all the isolates belonged to one single sequence type, ST- 4911 irrespective of the source of isolation (Table 3).

All the isolates cluster together in NCBI's pathogen detection pipeline (https://www.ncbi.nlm.nih.gov/Structure/tree/#!/tree/*Salmonella*/PDG000000002.2178/PDS000046205.3) indicating that they are highly related (averaging 1 pairwise SNP difference between all 9 isolates with a range of 0-4 SNPs). Two clinical isolates also fall into this cluster, collected outside of this study: one collected from the UK (maximum = 18 SNPs) and another from Canada (maximum = 5 SNPs) as shown in Figure 2.

Discussion

To the best of our knowledge, this is the first report on molecular characterization of *S*. Nigeria using WGS. This study confirmed the presence of *S*. Nigeria in pigs and poultry samples investigated. The isolation of *S*. Nigeria in this study is in agreement with the earlier studies in Nigeria by Fashae *et al.* [26] and Fagbamila *et al.* [27], about the isolation of *S*. Nigeria from pig and poultry farms respectively. This study reports the occurrence of 0.6 % (n = 9), this is higher when compared to Fagbamila *et al.* [27] with 0.3 % from poultry farms in Nigeria but low when compared to Fashae and Hendriksen [26] with 2.6 % (n = 8) obtained from pigs in Ibadan, South west Nigeria.

This highlights the economic importance of *S*. Nigeria in poultry, pigs, and public health in general. The differences in the occurrence rate may be due to several factors; differences in the farm location, the level of hygienic/biosecurity measures implemented on the farm, farm practice methods; intensive, semi-intensive, or extensive, and sources of food supplies to the farms investigated.

Contamination of poultry and pig farms with *Salmonella* have previously been attributed to low level implementation of hygiene and biosecurity measures on farms [35]. The presence of *Salmonella* in pigs and poultry in this study coupled with the previous reports

Figure 2. Phylogenetic tree of *S. enterica* Nigeria serovar showing the two clinical isolates from UK (917901) and Canada (PNCS006373).

by Fashae and Hendriksen [26] and Fagbamila *et al.* [27] presents the possibility of transmission of *S.* Nigeria to humans and animals, since most of the pig farms are semi - extensively managed and pig roams around to scavenge in poultry/pig farms, and on refuse dumps within the community, furthermore rodents and wildlife animals move from one farm to another in search of food leading to the possibility of cross contamination as earlier reported [17,36].

It is of significance to reiterate the importance of using global databases (Whole genome single nucleotide polymorphism (SNP) typing) to provide a phylogeny of *S.* Nigeria strains which helped to reveal the related strains on a global scale, two clinical isolates from the UK with 15 SNPs distance away and another from Canada with 05 SNPs. Surprisingly, no clinical isolates were linked to Nigeria, this may be due to WGS not being routinely performed in Nigeria, further evidence may be needed to confirm this assumption.

The low level phenotypic resistance obtained in this study could translate to the regulation of antimicrobials on farms and in feed as growth promoters in both poultry and pig husbandry as earlier speculated by Yang *et al.* [37], further investigation will be needed to confirm this assumption.

Similarly, the reported phenotypic resistance to tetracycline agreed with Fashae and Hendriksen [26] but contradicted our outcome with regards to sulphonamides and streptomycin susceptibility. Several studies on pig in some countries have consistently being in agreement with resistance to tetracycline as observed in some European countries as reported by Morar *et al.* [38].

The observed reduced susceptibility to fluoroquinolone (ciprofloxacin) due to the presence of *qnr*B19 gene, a plasmid mediated gene variant is in consonance with the outcome of similar studies from other geographical regions in Nigeria by Fashae and Hendriksen [26] and Jibril *et al.* [23].

Single mutation in *parC* (T57S) was detected in all the isolates, this mutation usually cannot independently confer quinolone resistance, *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 [39].

The detection of IncQ1, a plasmid associated with resistance to tetracycline, encodes *tetA* gene, and Col(pHAD28) plasmid which harbored quinolone resistance genes associated with decreased susceptibility to fluoroquinolones (ciprofloxacin) encoding *qnr*B19 gene were in agreement with similar studies from another region of Nigeria by Jibril *et al.* [23].

Salmonella harbored many virulence factors that play important roles in *Salmonella* infection by regulating host adaptability and pathogenicity. In *Salmonella*, the majority of virulence factor– encoding genes are located in regions distributed over the chromosome termed *Salmonella* pathogenicity islands (SPIs).

The detection of virulence genes from five SPIs, chromosomal, and fimbriae loci that were previously reported in other pathogenic *Salmonella*, showed that *S.* Nigeria has the potential to be pathogenic, further studies will be needed to confirm this hypothesis. Moreover, the detection of *invA* gene and other virulence factors that are essential for virulence in *Salmonella enterica* indicated the pathogenic potentials of *S.* Nigeria strain. The outcome of this study with regards to virulence genes is in agreement with Ammar *et al.* [40] that reported *invA* genes in Egypt.

Conclusions

This study provided valuable information on the antimicrobial resistance and virulence genes, sequence type (ST), plasmids profile and SNP analysis of *S.* Nigeria serovar from food-producing animals using WGS. We also reported for the first time, the genomic characterization of *S.* Nigeria serovar using WGS technique. The low level of resistance to majority of the commonly available antimicrobials used for clinical chemotherapy in human and veterinary practices is a positive development and requires continuous monitoring and enlightenment of the public on the danger of misuse of antimicrobials when it is not prescribed. Furthermore, the identification of multiple virulence genes that has the ability to cause disease (further studies is needed to confirm this hypothesis) is an important information for the public health scientists. Our findings provided a baseline information that will benefit future researchers and public health workers on the advantages of using WGS characterization for surveillance projects.

Limitations of this Study

The limitation of this study was the absence of any human isolates from Nigeria to compare globally as it was the case with the two isolates from UK and Canada. NCBI is a global database which can look at related isolates, until Nigeria starts sequencing clinical isolate it will be difficult to assess what is being transmitted from the food chain to human and vice versa.

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Authors' contributions

IAR, JAA and GA, were responsible for the conceptualization of the research. IAR, OAA, and AA, RET, RSH, were responsible for data collection and analysis. Writing of the original draft, review and editing were carried out by IAR, JAA, RET, RSH and GA.

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