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Molecular characterization of *Salmonella enterica* from poultry farms in Ilorin, north-central Nigeria

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Abstract

Poultry salmonellosis caused by *Salmonella enterica* is one of the most important bacterial diseases posing serious challenges to poultry production and human health worldwide. This study investigated the occurrence, serotypes, multilocus sequence types (MLSTs), antimicrobial resistance, plasmids, and 12 selected virulence genes of non-typhoidal *Salmonella* from poultry layer farms using whole-genome sequencing (WGS) methods. Two hundred cloaca swab samples were aseptically collected from four commercial poultry farms (layers) and transferred in sterile universal bottles on ice to the laboratory for analysis. Presumptive *Salmonella* isolates were detected with selective media and conventional biochemical tests. Serovars were confirmed by serotyping using the slide agglutination and SeqSero methods. Seven samples were positive for *Salmonella* consisting of *Salmonella* Typhimurium (*S. Typhimurium*) (n = 4), *S. Albany* (n = 2), and *S. Agama* with an occurrence rate of 3.5 % (7/200). Overall, 3 isolates showed the *parC* mutation expected not to cause resistance. Similarly, one *S. Typhimurium* isolate carried plasmid replicons of IncFIB(S)/IncFII(S) type without antimicrobial resistance genes. Three sequence types (STs); 19 (*S. Typhimurium*), 5317 (*S. Albany*), and 467 (*S. Agama*) were obtained. *Salmonella* Agama harboured 12 virulence genes, while *S. Typhimurium* and *S. Albany* harboured 11 virulence genes each. This study highlights the importance of *S. Typhimurium*, *S. Albany*, and *S. Agama* as major pathogens associated with poultry farms in Ilorin, north-central Nigeria. It equally provided baseline information on the serovar distribution, STs, resistance and the virulence gene profiles of all the serovars. Therefore, chickens can serve as a potential source of *Salmonella* transmission to humans, and this constitutes a potential health risk to the human population. Hence, there is a need for a specific *Salmonella* control program to be instituted as part of a national food safety strategy.

Keywords: Antimicrobial resistance, MLST, Nigeria, Poultry, *Salmonella* serovars, Virulence genes, WGS

Introduction

Over 2,600 *Salmonella enterica* serovars have been reported (Achtman *et al.*, 2012). However, the majority of infections are caused by a very limited number of serovars, which may differ from one country to another and over time (Hendriksen *et al.*, 2011; Van *et al.*, 2012). *Salmonella enterica* subsp. *enterica* serovars Enteritidis and Typhimurium are the most commonly reported serovars associated with human salmonellosis globally (Hendriksen *et al.*, 2011).

Salmonellosis in poultry is of global concern because it is commonly accompanied with morbidity, mortality, and significant economic losses (Abiodun *et al.*, 2014; Ahmed *et al.*, 2017). The disease can be transmitted vertically from parents to offsprings and horizontally on farms, thus, making its control a challenge. Infection by the horizontal route could be via infected litter, water, dust, faeces, feed, equipment, fomites, and rodents harbouring *Salmonella* (Raufu *et al.*, 2019). Therefore, humans are susceptible to contracting salmonellosis through the consumption of contaminated poultry products (Whiley & Ross, 2015).

The poultry sub-division of agriculture constitutes the most commercialized division in Nigeria, and the sector has transformed the lives of low-income farmers positively in a production system with little capital investment and low costs of technology. Furthermore, the sector has contributed an average of 454 billion tonnes of meat and 3.8 million eggs annually to the national economy with an approximate population of 180 million birds (Food and Agriculture Organization, 2018). *Salmonella* is among the major causes of human foodborne illnesses worldwide; however, little is known about its occurrence and genomic characteristics in most developing countries. Poultry and other food animals are regarded as common reservoirs of *Salmonella enterica*. Furthermore, undercooked poultry products have been identified as a major source of human infection with non-typhoidal *Salmonella* (Foley *et al.*, 2011). It has been reported that commonly isolated *Salmonella* serovars in a given location are usually among the most prevalent serovars associated with human infections, thus emphasizing the significance of *Salmonella* colonization of poultry farms to public health (Hendriksen *et al.*, 2011; Yang *et al.*, 2019). Therefore, the knowledge of *Salmonella* serovars distribution in food animals and humans is of value in investigating the course of *Salmonella* epidemiology and to identify serovars that cluster over time and space (Egualé, 2018).

The global burden of human diarrheal disease caused by *Salmonella* gastro-enteric infections is estimated at 93.8 million cases per year and 155,000 deaths (Majowicz *et al.*, 2010). Salmonellosis is specifically frequent among children in developing countries including Nigeria, where they often go unreported due to lack of a foodborne pathogens surveillance program. Apart from causing diarrheal disease, *Salmonella* has been implicated in invasive bloodstream infections, specifically in sub-Saharan Africa (Feasey *et al.*, 2012).

The emergence and spread of antimicrobial resistance among zoonotic *Salmonella* have become a public health concern in several developing countries due to the unregulated use of antibiotics for therapeutics and as poultry feed additives, which has led to the emergence of multidrug-resistant *Salmonella* (Ed-dra *et al.*, 2017). Furthermore, the development of antimicrobial resistance is often associated with the overuse and misuse of antimicrobials in veterinary and human medicine, especially in developing countries.

Resistance genes can spread from *Salmonella* in food animals to humans through mobile genetic elements (MGEs), such as plasmids, integrons and transposons that harbour resistance genes (Emond-Rheault *et al.*, 2020; Kudirkiene *et al.*, 2018). These mobile elements, especially the plasmids, may spread rapidly from multidrug-resistant clones of *Salmonella* within human and animal hosts (Carattoli, 2013; Hoffmann *et al.*, 2017).

In recent times, *Salmonella* strains with clinically important resistance to some agents like extended-spectrum cephalosporins and fluoroquinolones have been recovered from livestock (Li *et al.*, 2013). Previous reports have documented the relevance of the relatedness between nalidixic acid resistance and reduced susceptibility to fluoroquinolones in *Salmonella* (McDermott *et al.*, 2016). Therefore, it is imperative to investigate the mechanism of quinolone resistance in foodborne *Salmonella*, which could be traced to the occurrence of foodborne outbreaks and the subsequent problem of clinical therapy.

Different serovars are associated with different disease potentials, making serotype determination very necessary for epidemiological surveillance and disease assessment (Achtman *et al.*, 2012). Serotyping has been used for the epidemiological investigation of *Salmonella* by conventional serological methods based on White Kauffmann Le Minor scheme which classified *Salmonella* into over

2,600 serovars (Fagbamila *et al.*, 2017). Recently, sequence-based techniques such as Multi Locus Sequence Typing (MLST) and Whole Genome Sequencing (WGS) have been proposed as a replacement for serotyping to identify evolutionary and epidemiological relatedness (Ashton *et al.*, 2016; Robertson *et al.*, 2018). Such methods assign serotypes by using freely available in silico pipelines such as SeqSero, which utilizes surface antigen-encoding genes to predict serotypes (Zhang *et al.*, 2019).

Plasmid sequencing remains the main strategy for complete plasmid characterization. In recent times, plasmid extraction from whole-genome sequences is becoming increasingly common, and to date, a few bioinformatic tools have been developed for in silico detection and characterization of plasmids in whole-genome sequences (Arredondo-Alonso *et al.*, 2017; Orlek *et al.*, 2017).

The pathogenicity of *Salmonella* strains is related to various virulence genes harboured within the *Salmonella* pathogenicity islands (SPIs). These genes include *hilA* and *invA* that are located in SPI, which allows *Salmonella* to invade epithelial cells.

Furthermore, *Salmonella* outer proteins encoded by *sop* gene is also relevant to *Salmonella* virulence (Huehn *et al.*, 2010). Similarly, plasmid-encoded fimbriae genes (e.g. *pefA*) enhance the adhesion of

Salmonella to epithelial cells (Murugkar *et al.*, 2003). This study investigates the occurrence, serotypes, multilocus sequence types (STs), antimicrobial resistance, plasmids and virulence profiles of non-typhoidal *Salmonella* isolated from poultry farms in Ilorin, north-central Nigeria.

Materials and Methods

Study area/farms and sample collection

Four commercial poultry farms (layers) located at Egbejila, Eiyenkorin, and Lasoju communities in Ilorin, Kwara State, Nigeria (Figure 1), were visited for sample collection. The state lies on an elevation of 305 meters above sea level with a 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 border with the Republic of Benin in the west. The state has an annual rainfall range of 1,000 mm to 1,500 mm. Seasonal rain begins at the end of March and ends in early September. The dry season is from early October to early March.

Each farm was visited three times (once in two months) for sample collection, and each of the farms was visited on each sampling occasion.

The birds were managed intensively in battery cages, with each farm raising between 5,000 to 15,000 birds.

The average age of the flocks ranged between 65 and

80 weeks old (approximately 73 weeks old) at the start of our sampling. The farms obtained their day-old chicks from two different hatcheries located in a neighbouring state and obtained their feed supply from reputable feed mills. Twenty cloacal samples were collected from each farm on the first and second visits and ten samples each were collected on the final sampling day. Cloacal swabs were aseptically collected with a sterile

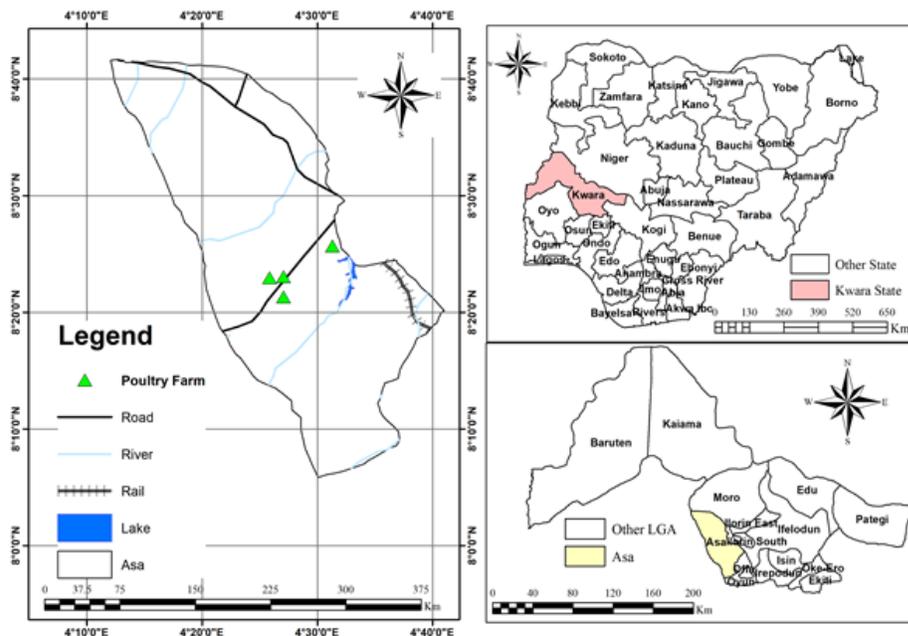


Figure 1: Map of Ilorin showing study area and farms for sample collection

commercial swab, immediately into a sterile sample bottle, and transported to the laboratory on ice for analysis.

Isolation and identification of Salmonella species

Samples were pre-enriched in buffered peptone water (Oxoid, Hampshire, UK) at a ratio of 1:10 sample to broth and incubated aerobically at 37°C for 24 hours. One milliliter of the pre-enriched broth culture was inoculated into 9.0 ml of Selenite-F broth swab (Fluka Biochemika, Steinheim, Germany) and incubated aerobically for 18 – 24 hours at 37°C. The broth was streaked on *Salmonella Shigella* agar (Laboratorios Britania, Buenos Aires, Argentina) and incubated for 18 – 24 hours at 37°C. Black colonies were sub-cultured on Xylose Lysine Deoxycholate (XLD) agar (Oxoid, Hampshire, UK) and incubated aerobically for 18 – 24 hours at 37°C. Presumptive *Salmonella* isolates on XLD with pink/red color and dark center were confirmed by standard biochemical tests and stored on nutrient agar slants for serotyping at the World Health Organization (WHO) National *Salmonella* and Shigella Center, Bangkok, Thailand according to the Kauffmann-White Scheme as previously described (Raufu *et al.*, 2009).

Whole Genome Sequence (WGS) data collection

Two hundred *Salmonella* isolates were shipped on nutrient agar slants to U.S. Food and Drug Administration, College Park, Maryland, USA, for WGS analysis. According to the manufacturer's instructions, bacterial DNA was extracted from overnight cultures using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). Sequencing libraries were constructed with 0.2 ng/μl of prepared DNA immediately uploaded to FDA's GenomeTrakr database (Allard *et al.*, 2016), hosted within NCBI Pathogen Detection (NCBI Resource Coordinators, 2017) under the bioproject PRJNA186035. The SRA accession numbers and assembly statistics of all the *Salmonella enterica* serovars are shown in Table 1.

Whole Genome Sequence (WGS) analysis

Raw data were subjected to genome assembly using SPAdes version 3.8 (Bankevich *et al.*, 2012), and genome annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al.*, 2016). *Salmonella* serotyping by whole-genome sequencing was predicted with the SeqSero version 1.0 software tool (Zhang *et al.*, 2015). 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 (Seemann, 2015) and a modified version of the PlasmidFinder database (Zankari *et al.*, 2012). Predicted resistance phenotypes were assigned by using the determinants detected and the ResFinder and PointFinder drug keys developed by the Centers for Disease Control and Prevention (Feldgarden *et al.*, 2019).

Multilocus sequence types (MLST) were determined by WGS based on sequencing of the seven housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. The contig sequence files were uploaded to the Centre for Genomic Epidemiology MLST database. In silico multilocus sequence typing (MLST) data were extracted from the Center for Genomic Epidemiology online tool (Larsen *et al.*, 2012) to assign sequence type (ST) to each study isolate based on the set of alleles derived from the aforementioned seven loci. The virulence genes present in the genome were extracted from the Public Health Agency of Canada website (Chen *et al.*, 2016).

Results

Analysis of genomic data obtained from WGS showed the total lengths of genomes ranged from 4.6 to 4.9 Mb, G + C average content ranges from 51.70 to 52.20 %, the numbers of contigs range from 32 to 74. The numbers of coding genes, noncoding RNAs (ncRNAs), tRNAs, (CRISPR) arrays, genome coverage, number of CDS (total), rRNA (complete) and the contig N50 ranges from 4,266 to 4,674, 10 to 14, 60 to 73, and 2 to 3, 64x to 131x, 4, 350 to 4,765, 3 (58) to 6 (58) and 175, 261 to 303, 664, respectively (Table 1).

Out of 200 samples, seven samples were positive for *Salmonella* (Table 2). Three serovars were represented: *Salmonella* Typhimurium (*S. Typhimurium*) (n=4), *S. Albany* (n=2), and *S. Agama* (n=1). Three sequence types (STs) were obtained from WGS analysis, these are ST-19, 467, and 5317 exhibited by *S. Typhimurium*, *S. Agama*, and *S. Albany* respectively (Table 2).

Genotypic antimicrobial susceptibility results showed three isolates; *S. Albany* (n = 2) and *S. Agama* (n = 1) to have a single T57S mutation in the *parC* gene alone. All the four *S. Typhimurium* strains lacked resistance determinants but one encoded IncFIB(S)/IncFII(S) plasmid replicons (Table 2).

Twelve virulence genes were identified across several virulence loci consisting of SP-1, 2, 3, 5, 11, and chromosomal encoded *sifA* gene. The genes investigated include: SP-1; *InvA*, *SipB*, *prgH*, *avrA*, SPI-2; *ssaR*, *ssaB/spiC*, SPI-3; *misL*, *mgtB*, SPI-5; *PipB*, *sopB*, SPI-11; *CdtB*. Only *Salmonella* Agama harbored each of the 12 identified virulence genes (Table 3).

Table 1: Assembly statistics of *Salmonella enterica* serovars isolated from poultry farms in Ilorin, Nigeria

Sample ID	<i>Salmonella</i> serovar	GenBank accession No.	Biosample accession No.	Genome size					No. of CDS (total)	No of coding genes	rRNA (complete)	tRNA	nc RNA	CRISPR Arrays
				Length (bp)	No. of Contigs	Coverage (x)	G +C (%) contents	Contig N50						
CFSAN083238	Typhimurium	GCA_006007725.1	PDS000039015.6	4,859,818	67	131x	51.70%	178,292	4,725	4,632	3 (5S)	61	14	3
CFSAN083242	Typhimurium	GCA_006147495.1	PDS000039015.6	4,858,370	72	94x	51.80%	175,261	4,734	4,642	3 (5S)	64	11	2
CFSAN083253	Typhimurium	GCA_006147355.1	PDS000039015.6	4,890,353	64	102x	51.90%	181,079	4,765	4,674	3 (5S)	62	12	3
CFSAN083257	Typhimurium	GCA_006081075.1	PDS000039015.6	4,829,700	66	91x	52.00%	175,364	4,688	4,595	3 (5S)	60	11	3
CFSAN083241	Albany	GCA_006080655.1	PDS000038995.2	4,680,993	42	102x	51.60%	237,090	4,471	4,370	5 (5S)	61	10	2
CFSAN083276	Albany	GCA_005902125.1	PDS000038995.2	4,656,289	32	106x	52.20%	303,664	4,532	4,532	6 (5S)	73	10	2
CFSAN083240	Agama	GCA_006294055.1	PDS000039006.4	4,586,567	47	103x	51.70%	214,188	4,350	4,266	4 (5S)	64	11	2

Table 2: Serotypes, antimicrobial resistance, plasmids and MLST types of *Salmonella* serovars from poultry farms in Ilorin, North-central Nigeria

Sample ID No.	NCBI-SRA SRA Run No.	Primary Taxonomy Hit	Predicted Serotype	SeqSero2(JC)	MLST	Resistance Determinants	Plasmids	Anticipated Resistance
CFSAN083238	SRR9163976	<i>Salmonella enterica</i>	Typhimurium	Typhimurium	19	None	None	None
CFSAN083242	SRR9164963	<i>Salmonella enterica</i>	Typhimurium	Typhimurium	19	None	None	None
CFSAN083253	SRR9164982	<i>Salmonella enterica</i>	Typhimurium	Typhimurium	19	None	IncFIB(S) / IncFII(S) [‡]	None
CFSAN083257	SRR9164977	<i>Salmonella enterica</i>	Typhimurium	Typhimurium	19	None	None	None
CFSAN083241	SRR9163974	<i>Salmonella enterica</i>	Albany	Albany	5317	<i>parC</i> (T57S)	None	None
CFSAN083276	SRR8266852	<i>Salmonella enterica</i>	Albany	Albany	5317	<i>parC</i> (T57S)	None	None
CFSAN083240	SRR9163977	<i>Salmonella enterica</i>	Agama	Agama	467	<i>parC</i> (T57S)	None	None

Key: ‡ = single plasmid with two replicons

Table 3: Virulence genes of *Salmonella* serovars from cloaca swabs of chickens from poultry farms in Ilorin, north-central Nigeria

Virulence loci	Gene	<i>Salmonella</i> serotypes						
		Typhimurium	Agama	Albany	Typhimurium	Typhimurium	Typhimurium	Albany
SPI-1								
	<i>InvA</i>	+	+	+	+	+	+	+
	<i>SipB</i>	+	+	+	+	+	+	+
	<i>prgH</i>	+	+	+	+	+	+	+
	<i>avrA</i>	+	+	+	+	+	+	+
SPI-2								
	<i>SsaR</i>	+	+	+	+	+	+	+
	<i>ssaB/spiC</i>	+	+	+	+	+	+	+
SPI-3								
	<i>misL</i>	+	+	+	+	+	+	+
	<i>mgtB</i>	+	+	+	+	+	+	+
SPI-5								
	<i>PipB</i>	+	+	+	+	+	+	+
	<i>sopB</i>	+	+	+	+	+	+	+
SPI-11								
	<i>CdtB</i>	-	+	-	-	-	-	-
Chromosomal								
	<i>sifA</i>	+	+	+	+	+	+	+

Discussion

Salmonella is an important zoonotic pathogen, and its occurrence in animals constitutes a potential threat to public health. In this study, a low-level occurrence of *Salmonella* was observed in poultry farms investigated. The isolation rate of *Salmonella* from this study is lower when compared to similar studies in Maiduguri, northeastern Nigeria (Raufu *et al.*, 2013), Ibadan, southwestern Nigeria (Fashae *et al.*, 2010) and from commercial poultry farms in Nigeria (Fagbamila *et al.*, 2017). These variations may be due to low sample size, types of management practices and the level of biosecurity measures implemented on the farms. Similar studies from other African countries like Ghana, Uganda, and Ethiopia, and Algeria have been reported by Andoh *et al.* (2016); Odoch *et al.* (2017); Eguale (2018) and Elgroud *et al.* (2009), respectively, suggesting chickens are important reservoirs of *Salmonella* in poultry.

The lower occurrence commonly observed in the European member countries is attributable to the strict implementation of specific control programs such as the strict hygiene and biosecurity practices on the farms. Furthermore, poultry farms are not located in close proximity to one another and movement of personnel and farm equipment between poultry farms is reduced in more developed countries. Such practices are difficult to implement in most developing countries like Nigeria. The low *Salmonella* prevalence obtained in this study is likely due to a small sample size taken from medium-sized poultry farms studied. The prevalence may be greater among larger farms because it is more difficult for the farmers to adhere to strict farm bio-securities and good farm management practices in such establishments (Adesiyun *et al.*, 2014).

Three *Salmonella* serotypes comprising of *Salmonella* Typhimurium, *S. Albany*, and *S. Agama* were obtained

in this study, and *Salmonella Typhimurium* was the most prevalent serotype (4/7). *Salmonella Typhimurium* is a common serovar in chickens and most frequently causes foodborne disease worldwide (Freitas *et al.*, 2010; Hendriksen *et al.*, 2011). The serovar is most commonly involved in salmonellosis outbreaks and has been reported by other researchers in Nigeria (Orji *et al.*, 2005; Fasura *et al.*, 2012). Similarly, it has been documented from poultry in Trinidad and Tobago (Adesiyun *et al.*, 2014) and in Algeria (Jakirul *et al.*, 2016). These observations contradicted Fagbamila *et al.* (2017), who described the role of *S. Typhimurium* in the Nigerian poultry industry as marginal.

Interestingly, one *S. Typhimurium* from this study shared similar features with one of the strains reported by Kudirkiene *et al.* (2018) in Ghana, they both belonged to sequence type, ST-19 and carried plasmid replicons IncFII(S)/IncFIB(S) and both lacked resistance genes. But in contrast, the strain from Ghana showed resistance to amino-penicillin and sulphometaxazole classes of antimicrobial agents, while the strain from this study lacked resistance to any antimicrobial agent.

Salmonella Albany is a rarely reported serotype from poultry in Nigeria; it is commonly associated with poultry contamination in Malaysia and other Asian countries as reported by Jeon *et al.* (2016) and Jajere *et al.* (2019). *Salmonella* Agama was first reported in Ibadan, southwest Nigeria from the faeces of Agama lizard (Collard & Sen, 1960). Recently, however, it is frequently isolated from human and food animals in Nigeria and Africa in general (Orji *et al.*, 2005). This study contradicted previous studies that reported *S. Kentucky* as the most prevalent serovar in poultry and humans in Nigeria (Muhammad *et al.*, 2009; Raufu *et al.*, 2013). The observed low level of resistance to the majority of the antimicrobials in this study, including those critical for the treatment of salmonellosis in humans such as nalidixic acid and ciprofloxacin, is encouraging. This level of resistance may be the result of cautious antimicrobial utilization at recommended doses for chemotherapeutic and prophylactic control of bacterial infections on poultry farms investigated. These findings contradicted the report of Fashae *et al.* (2010), who reported a high level of resistance to nalidixic acid and reduced susceptibility to ciprofloxacin. However, our sample size in this study would limit such comparison to a certain limit.

Virulence genes are essential for *Salmonella* pathogenicity. These genes are vital for the survival of *Salmonella* during the invasion of the intestinal epithelial cells, replication within macrophages,

adherence to, and systemic infection of the host cells. *Salmonella* pathogenicity islands (SPIs) are chromosomal regions that harbor such genes, and these are involved in *Salmonella* invasion of and survival within host cells (Ahmed *et al.*, 2018).

The detection of several virulence genes across several virulence loci in conjunction with the identification of chromosomal genes (chromosomal loci) demonstrated that isolates from this study are potential pathogens. Moreover, the identification of *invA* gene is essential for virulence in *Salmonella* and is regarded as a standard for *Salmonella* detection (Mezal *et al.*, 2013). Thus, virulence gene screening corroborated the pathogenic potential of all the isolates.

In this study, MLST was utilized to determine the sequence types; the sequence types obtained for *S. Typhimurium* was ST-19, which is similar to findings reported by Harb *et al.* (2018) in Iraq and Jain *et al.* (2018) in India. Similarly, Zhao *et al.* (2017) obtained ST-19 as the most frequent genotype that was recovered in pigs and belonged to *S. Typhimurium*. The MLST database has shown ST-19 to be the most widespread genotype of *S. Typhimurium*; however, it is noteworthy to mention that another strain of *S. Typhimurium*, ST-313, has been predominant in Africa, especially in Nigeria, Kenya, Malawi, Zimbabwe, Uganda, and Ethiopia (Kingsley *et al.*, 2009) but was not detected in this study. *Salmonella* Albany is a rare serotype from poultry in Nigeria, and the detected sequence type was ST-5317. This contradicted the *S. Albany* ST-292 reported in Colombia from broiler chickens by Castellanos *et al.* (2018), who indicated the presence of more than one circulating clone. Finally, the sequence type obtained for *Salmonella* Agama was ST-467.

This study highlights the relative importance of *S. Typhimurium*, *S. Albany*, and *S. Agama* as pathogens associated with poultry farm contamination in Ilorin, the north-central region of Nigeria. It equally provided baseline information on the serovar distribution, sequence types (STs), antimicrobial susceptibility, and the virulence gene profiles of the identified serovars. Furthermore, within the context of this study, the absence of resistance to fluoroquinolones and nalidixic acid may correlate with responsible antimicrobial administration on the farms or an overall low level of antimicrobial resistance on the farms in the study area. The isolation of *Salmonella* on the farms emphasized the need for heightened awareness by national and state authorities to institute measures to monitor and limit

the spread of *Salmonella* by instituting effective biosecurity measures on the farms.

Major limitations observed in this study are the small sample size and the small number of farms investigated. However, these gave a narrow interpretation of the isolates collected using the described methodology, which was appropriate for *Salmonella* isolation and can be utilized for further studies. Therefore, other studies with a larger sample size covering more poultry farms and spread over several regions are recommended.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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