



Prevalence and Characterization of Salmonella Isolates from Poultry Farms in Ilorin, Nigeria

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Abstract

Poultry birds are very important source of essential proteins in developing countries, but also play an important role in transmission of Salmonella to human and nonhuman. Exposure to this pathogen also occurs through use of poultry droppings as manure for crop and vegetable production. This study investigated prevalence of Salmonella in feces of poultry farms in Ilorin, Nigeria. Salmonella isolated were further characterized by molecular method. Fecal samples were cultured in pre-enrichment medium, Selenite F medium and finally sub-cultured on Salmonella-Shigella agar. None lactose fermenting colonies with black center were picked for presumptive identification using biochemical tests and confirmed by serological test by method previously described. Molecular characterization was carried out using 16S rRNA gene sequencing following standard procedure. Out of 170 samples collected and examined, 8 (4.7%) gave biochemical characteristics that resembled Salmonella, but only 6 (3.5%) were confirmed as Salmonella by polyvalent antisera. Molecular characterization revealed that serovars isolated were Salmonella Enteritidis 3 (1.8%) and Salmonella Paratyphi 3 (1.8%). Phylogenetic tree constructed by neighbor-joining method as derived from analysis of the 16S rRNA gene sequences showed close relationship to Salmonella Paratyphi strain FB0015 16S ribosoma RNA gene partial sequence to our Salmonella Paratyphi isolates. The detection of 3.5% prevalence rate of Salmonella serovar from feces of laying poultry birds my serve as potential source of transmission of this pathogen to human through chicken meat, egg or use of poultry dropping as manure in crop or vegetable production.

Keywords: Salmonella, Poultry, Serovars, Genetic relatedness, Faeces.

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1. Introduction

Salmonella is a ubiquitous and hardly bacteria capable of surviving in dry and wet environments. It is a prominent member of the family Enterobacteriaceae that consists of more than 2,500 different strains [1]. While a few serotypes are host specific and can reside in only one or few animal species, others have a wide range of hosts. Some Salmonella have been considered as one of the most important food-borne pathogen around the world [1, 2].

Salmonellosis is often associated with gastroenteritis; characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting. The organism can seep into the blood stream causing bacteremia and typhoid fever. Salmonellosis is one of the most common and widely distributed foodborne diseases, with tens of millions of human cases occurring worldwide per annum [1]. In tropics especially in sub-Saharan Africa, non-typhoidal salmonella has consistently been reported as leading causes of bacteremia and gastroenteritis among elderly, malnourished children, infants and immunocompromised individuals [3].

Poultry farming is one of the major sources of meat protein in Nigeria, where large scale farms with thousands of birds exist, others have few hundred birds. They serve as major source of animal protein either as meat or egg. Contamination of poultry birds arise from either contamination of feeds or water resulting in contamination of feces, litters, eggs and chicken parts which may be source of human acquisition of the infection. The sources and mode of transmission of Salmonella in developing countries are poorly understood and may be difficult to trace, due to lack of coordinated national surveillance system, poor supervision of farms and animal feed by authorities concerned [4].

While poultry droppings are very useful as manure for crop and vegetable production, they can also serve as vehicle of spread of infectious diseases to human. Previous studies on Salmonella from poultry droppings in Nigeria are limited to certain geographic region or state of the federating units and may not be a representative of other regions or states. This study aimed at bridging this gap, and also at investigating prevalence of Salmonella species in the feces of poultry birds in Ilorin, Kwara State Nigeria.

Previous studies have shown that *Salmonella* species have different disease syndromes and host specificity according to their antigenic profile [3]. Therefore it is expedient to discriminate *Salmonella* serovars isolates from each other. This will ensure that each serovar can be tracked in case of epidemics, outbreak detection or general surveillance. For this reason molecular characterization was further carried out after cultural isolation, biochemical identification and serologic confirmation in this study.

2. Materials and Methods

2.1. Sample Collection

A total of 170 poultry fecal samples were collected from three medium size farms with appropriately one thousand (1,000) to one thousand two hundred (1,200) laying birds in an intensive confinement. The samples were collected within a period of three months (July to September, 2016). Ninety (90) samples were collected from Kwara State University farm (Moro LGA), and forty (40) each from two farms in Tanke (Ilorin South LGA), identified as Tanke A and Tanke B farms. Representative of freshly passed fecal samples were randomly collected with a spoon attached to the cap of sterile plastic bottles. Approximately 7-8 grams of the samples were collected and arranged in ice pack glostyle bag and transported to Microbiology laboratory of Kwara State University, Malete.

2.2. Sample Analyses

About 4 grams of fecal sample was inoculated into pre-enriched 50 mL buffered peptone (Lab M Ltd, Quest Park, Bl97JJ, UK) in a 100 mL flask, a modification of method described by Antunes, et al. [5]. This was incubated at 37°C for 18 hours in ambient air. One milliliter was sub-cultured into 10 mL of Selenite F broth (Lab M Ltd, Topley House, BL96AS, UK) and re-incubated at 37°C for another 18 hours. It was further sub-cultured onto Salmonella-Shigella and MacConkey agar (Lab M Ltd, Topley House Bl96AS, UK) for another 18 hours at 37°C. None lactose fermenting colonies with black center were picked for presumptive identification using biochemical tests and confirmed by serological test using polyvalent antisera (Oxoid Ltd, UK) as previous described by both Forbes, et al. [6] and Barrow and Feltham [7].

Salmonella isolates were characterized by molecular method using 16S rRNA gene sequencing. The genomic DNA of each isolate was extracted using commercial DNA isolation kit (Real Biogenomics, UK) following the instruction manual of the manufacturer. The 16S rRNA gene of the target isolates were amplified by using universal eubacterial primer as reported by Heddi, et al. [8].

Table-1. Base sequences of 16S rRNA gene primers

Name of the Primer		Sequence (5' to 3')
16S rRNA gene	F	AGA GTT TGA TCA TGG CTC AG
	R	TAC CTT GTT ACG ACT TCA CC

Source: Sigma, Pvt. Ltd

The amplified PCR products were resolved by electrophoresis using one percent agarose gel in Tris acetate EDTA buffer. Agarose gel was mixed with ethidium bromide (0.5 µg/ml) before pouring. One kb DNA ladder (Bangalore Genei) was used as a marker. The gel was run at run at 120V for 45 minutes using Bangalore Genei power Pac System (USA). Ethidium bromide stained gel was viewed images captured using gel documentation system (Alphalmager, 2200, Alpha Infotech Corporation, USA).

2.3. Agarose Gel Electrophoresis of PCR Products

The amplified PCR products were resolved by electrophoresis using 1 per cent agarose gel in 1X Tris acetate EDTA buffer (2 M Tris base, 57.10 ml acetic acid and 0.5 M EDTA, pH 8.0, 50 X). Agarose gel was mixed with ethidium bromide (0.5 µg/ ml) before pouring. 1 kb DNA ladder (Bangalore Genei) was used as a marker. The gel was run at 120V for 45 minutes using Bangalore Genei Power Pac system. The ethidium bromide stained gel was

viewed and image captured using gel documentation system (Alphamager 2200, Alpha Infotech Corporation, USA).

2.4. Sequencing and Data Analysis

PCR products of 16S rRNA gene of six efficient bacterial isolates obtained through amplification with specific primer were freeze dried in a lyophilizer (CHRIST ALPHA I-2LD) sequenced using same upstream and downstream primers used for the amplification of 16S rRNA gene (Ocimum Biosolutions, Pvt. Ltd.).

2.5. Nucleotide Sequence Analysis

The 16S rDNA sequences of different bacterial isolates were BLAST (Basic local alignment search tool) searched against the sequences of 16S rRNA of bacterial isolates available in the Genbank Nucleotide Database Sequences with more than 98 % identify with a GenBank sequence were considered to be of the same species as the highest score-matching sequence on the public sequence databases as in Altschul, et al. [9] for sequence comparison. The sequences were aligned by Clustal W program using website [10] and pair wise per cent nucleotide sequence similarities between isolates 3 and 3 isolates and other selected bacterial sequences from NCBI database were determined. Phylogenetic analysis was performed with neighbor-joining method using program in Molecular Evolutionary Genetics Analysis (MEGA) version 5.1 [11].

3. Results

Out of 170 fecal dropping samples collected and cultured for Salmonella, 8 were presumptively identified as Salmonella by biochemical methods and 6 (3.5%) of were confirmed with serologic method to be *Salmonella* serovar. Samples distribution and Salmonella isolation was as presented in Table 2.

Table-2. Showing distribution of Salmonella isolates among the three farms

	No of samples (%)	No Positive for Salmonella (%)
KWASU	90 (52.9)	3 (3.3)
Tanke A	40 (23.5)	2 (5.0)
Tanke B	40 (23.5)	1 (2.5)
Total	170 (100)	6 (3.5)

Source: This was generated from sample collection sources

The molecular characterization of nucleotide sequence analysis revealed that the strains isolated were 3 *Salmonella* Enteritidis and 3 *Salmonella* Paratyphi in the 99% and 100% homogeneity respectively.

3.1. Nucleotide Sequence Analysis

Nucleotide sequence analysis of test isolates using clustalW program revealed that isolates 3 showed maximum homology (100%) with *Salmonella* Paratyphi strain FBD 0015 (EF 643617.1), isolate 3 showed homology (99%) with *Salmonella* Enteritidis strain E6 (EU118106.1). The test bacterial isolates clustered with members of the genus *Salmonella*

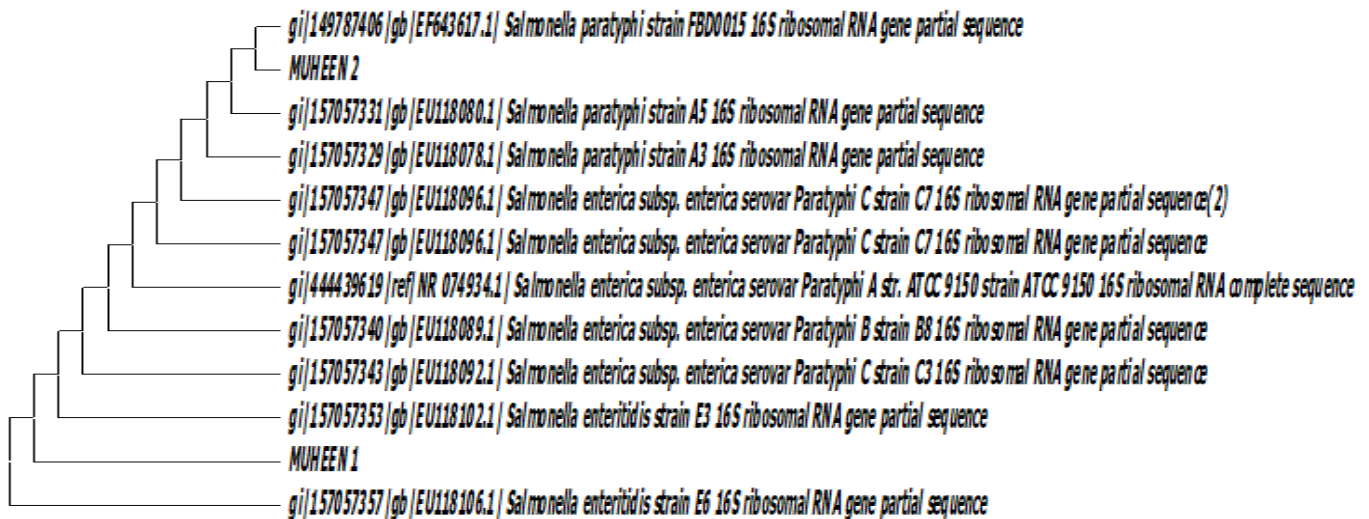


Figure-1. Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16S rRNA gene sequences of native isolates and related sequences obtained from NCBI.

Source: GenBank: Lus AE006468 NCBI, ncbi.nlm.nih.gov

4. Discussions

The prevalence rate of Salmonella detected was 3.5% among three intensive poultry farms studied. The prevalence may be regarded as low compared with prevalence rates of 18.4% reported in Kaduna, Nigeria by Mbuko, et al. [12] 9.4% reported at Jos, Nigeria by Okwori, et al. [13] and 11% of fowls reared at intensive poultry farms in Ibadan, Nigeria by Fashae, et al. [4]. The low incidence in this study might be attributed to high standard of hygiene employed by these farmers and record of constant visit to the farms by veterinary doctors. The farms also have boreholes as source of water supplied that may minimize fowl infections through water source.

In this study two species of Salmonella were isolated, *Salmonella* Enteritidis and *Salmonella* Paratyphi. In Africa, as well as elsewhere in the world, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common causes of human salmonellosis [14]. Interestingly, only *Salmonella* Enteritidis was recovered from the fecal dropping of laying birds in our study, but *Salmonella* Typhimurium was not detected. However, in similar study in

Burkina Faso, the main serotype found in poultry, animal and human fecal samples were *Salmonella* Typhimurium and *Salmonella* Muenster (from all the animal species) [15].

Previous study on PFGE provides valuable phylogenetic relationship inference for *Salmonella* at serovar level [16]. In this study, phylogenetic tree constructed by neighbor-joining method as derived from analysis of the 16S rRNA gene sequences showed close relationship among *Salmonella* Paratyphi strain FB0015 16S ribosomal RNA gene partial sequence to our *Salmonella* Paratyphi isolates. Similarly our *Salmonella* Enteritidis is closely related to *Salmonella* Enteritidis strain ES 16S ribosomal RNA and *Salmonella* Enteritidis strain E6 16S ribosomal RNA obtained from NCBI (Figure 1). This genetic relatedness was 99% and 100% of *Salmonella* Enteritidis and *Salmonella* Paratyphi to our isolated *Salmonella* Enteritidis and *Salmonella* Paratyphi respectively.

Salmonella Enteritidis and *Salmonella* Paratyphi are readily transmitted from egg and poultry meat to human with health consequences Kingsley, et al. [17]. Report by Acha and Szyfres [1] stated that 60-80% of all Salmonellosis cases are not recognized as part of a known outbreaks and classified as sporadic cases or are not diagnosed as such at all.

Hygienic methods to be employed in poultry products handling to avoid transmission of infectious agents should include proper hand washing, personal hygiene of workers, properly boiling of poultry meat and egg before consumption. The use of poultry droppings as manure for improving crop yields especially vegetables that may be consumed raw should be discouraged. This is because *Salmonella* are hardy are able to survive several weeks in dry environment and several months in moisture condition and in water [1].

5. Conclusion

This study revealed detection of *Salmonella* Enteritidis and *Salmonella* Paratyphi in poultry drooping of intensive farms in Ilorin, Nigeria, however with low prevalence. The isolated strains were genetically related to human strain. Carriage of *Salmonella* in poultry dropping is a potential source of acquisition of infection by human. We therefore suggest systematic surveillance of poultry farms by health experts and discourage in use of poultry droppings as manure for vegetables that are not cooked before consumption.

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