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## Research Article

# Occurrence of *Salmonella* and their Antimicrobial Susceptibility Pattern Associated with Poultry Accessories in Maiduguri, Nigeria

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## Abstract

**Background and Objectives:** The objective of the study was to determine the occurrence of *Salmonella* and their antimicrobial susceptibility pattern in poultry feeds, faeces, drinking water and litters sampled from Teaching and Research Poultry Farm and Wilson Poultry Farm located in Maiduguri the capital city of Borno State, Nigeria. **Materials and Methods:** The study design was a cross sectional study. A total of 120 samples were collected with 60 samples each from the two locations. Fifteen samples each from poultry faeces; drinking water, feeds and litters were collected using convenience sampling technique. About 10 g each of the samples from faeces, feeds and litters were weighed. Similarly, 10 mL of water sample were equally measured. Faeces, feeds and litters were collected in plastic bags and drinking water in aseptic sample bottles and then labeled properly. Isolation and identification of *Salmonella* were performed according to standard bacteriological protocol. Susceptibility testing of *Salmonella* isolates to 10 different antimicrobial agents was carried out using disc diffusion method on Muller-Hinton agar. Data generated were analyzed using descriptive statistics with Microsoft Excel version 2010. **Results:** The overall prevalence of *Salmonella* in poultry samples from Teaching and Research Poultry Farm was found to be 31.67%. The highest prevalence of 53.33% was obtained from faeces followed by litters (33.33%) and 20.00% was recorded for samples obtained from feeds and drinking water, respectively. The overall prevalence of *Salmonella* in poultry samples from Wilson Poultry Farm was found to be 46.67%. Higher prevalence of 80.00% was obtained for samples from litters, followed by faeces (66.67%), while 26.67 and 13.3% were associated with drinking water and feeds, respectively. The resistance and sensitivity pattern were displayed by 24 *Salmonella* isolates to 10 anti-microbial agents. Septrin (SXT), Ampicillin (PN) and Ceporex (CEP) had the highest resistance of 5 (10.64%), 4 (8.51%) and 4 (8.51%) to *Salmonella* isolates, while Ciproflox (CPX) showed no resistance (0.00%). The *Salmonella* isolates recorded higher sensitivity to Ciproflox (CPX), 12 (25.53%), Tarivid (OFX), 10 (21.28%), Augmentin (AU) and Reflacine (PEF), 9 (19.15%). **Conclusion:** From the results of this study, it has been demonstrated that the *Salmonella* present in poultry feeds, faeces, drinking water and litters may pose a risk to humans who are poultry consumers.

**Key word:** Occurrence, *Salmonella*, antimicrobial, susceptibility, poultry, Nigeria

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The members of the genus *Salmonella* are Gram-negative, motile, facultative anaerobic bacilli belonging to the family Enterobacteriaceae<sup>1</sup>. *Salmonella* comprised of two central species; *Salmonella enterica* and *Salmonella bongori*. *Salmonella* is a leading cause of food-borne mortality and morbidity in United States and other parts of the world<sup>2</sup>.

The occurrence of *Salmonella* species has become one of the major health challenges not only in the study area but in several parts of Nigeria<sup>3</sup>. Salmonellosis ranges in severity from self-limiting gastroenteritis to septicaemia<sup>4</sup>. The severity of the salmonellosis depends heavily on host susceptibility and the virulence of the serovars<sup>1</sup>. It is characterized clinically by one or more of the three major syndromes, septicaemic, acute and chronic enteritis which infect both humans and animals with millions of illness reported worldwide<sup>5,6</sup>. Salmonellosis is one of the leading causes of bacterial gastroenteritis in humans and is responsible for over 1.4 million illnesses annually in the USA<sup>7</sup>. Although, *Salmonella* species also cause clinical diseases in a variety of animal species, many domestic and wild animals become colonized and shed these bacteria in their faeces with apparent sign of illness. If ingested either through direct exposure to faeces or through faecal contamination of food or water, dominant *Salmonella* serovars can subsequently cause disease in humans and other animals<sup>8</sup>.

The infected birds may serve as transport vehicle for transmission of *Salmonella* species to humans through the consumption of poultry meat and eggs. Similarly, multi-drug resistance of certain pathogenic strains of *Salmonella* to antimicrobial agents may serve as a public health risk to humans. Antibiotic resistance among *S.* species is a well confirmed phenomenon. Animals are the primary source of zoonotic *Salmonella*<sup>9</sup>. The effective understanding of poultry *Salmonella* and information on their identifiable characteristics will enable significant improvement in their control strategies. Therefore the study was conducted to determine the occurrence of *Salmonella* in poultry associated with their accessories and to demonstrate the antimicrobial susceptibilities of the *Salmonella* isolates to ten different antimicrobial agents.

## MATERIALS AND METHODS

**Study area:** The study area is Maiduguri, Borno state which is located in the north-eastern, arid zone of Nigeria. It has an area of about 69,436 km<sup>2</sup> and lies within latitude 11°50' 46" N

and longitude<sup>10</sup> 13°08' 29" E. It is found within the Savannah with low records of rainfall. The area is in the tropical continental north with dry period of 4-8 months, October-May, followed by a short rainy season from late June to early October. The Borno state has boundaries with Chad to the northeast, Cameroon to the east and Adamawa state to the southwest. Agriculture and livestock farming is the mainstay of the State economy.

**Study design:** The study design was a cross sectional study.

**Duration of the study:** The duration of the study was for a period of 9 months beginning from October-June, 2018.

**Sampling and sample collection:** A total of 120 samples were collected from Teaching and Research Poultry Farm, University of Maiduguri and Wilson Poultry Farm Fori Ward, Maiduguri. Sixty samples were collected from each of the two locations. Fifteen samples each from poultry faeces, drinking water, feed and litter were collected using convenience and not convenient technique. About 10 g each, of the sample from faeces, feeds and litters were weighed. Similarly, 10 mL of water sample were equally measured. Faeces, feeds and litters were collected in plastic bags, while drinking water was collected in aseptic sample bottles and were labeled accordingly, kept in a cool box containing ice blocks and then transported to the Veterinary Microbiology Laboratory, Department of Veterinary Microbiology, University of Maiduguri, where they were processed immediately.

**Isolation and identification of *Salmonella*:** About 90 mL of normal physiological saline was added to the 10 g of each sample and was mixed thoroughly about sieved. One milliliter of each sieved (or filtered) sample solution was inoculated into Selenite F broth and incubated at 37°C for 18-24 h. A loopful of the broth culture was sub-cultured onto Deoxycholate citrate agar (DCA), incubated at 37°C for 24-48 h. This was subsequently sub cultured onto nutrient agar slants, incubated for 18-24 h and then stored at 4°C until required. The Gram's-staining techniques was performed on pure black colonies of *Salmonella*<sup>11</sup>. The biochemical characterization was carried out using Triple Sugar Iron (TSI), Simmon Citrate, Urease and Indole tests to check for phenotypic changes produced by *Salmonella* within the media<sup>12</sup>.

**Antimicrobial susceptibility testing of *Salmonella* isolates:** Susceptibility testing of *Salmonella* isolates to 10 different antimicrobial agents was carried out through

disc diffusion method, to determine their antimicrobial susceptibility pattern. The antibiotics include: Ciprofloxacin, Streptomycin, Pefloxacin, Amoxicillin, Ofloxacin, Apramycin, Ceftiofur and Cefotaxime. The isolate of *Salmonella* were subcultured into Muller-Hinton broth (Oxoid-CMO405) and then incubated at 37°C for 24 h. Serial dilution was carried out in the ratio of 2:4 of the broth and distilled water to reduce the degree of turbidity of the broth culture. About 1 mL was dispensed onto Muller-Hinton agar and the excess was discarded. Antibiotic discs were applied aseptically to the surface of the plate. This was incubated at 37°C for 24 h under aerobic condition to observe for zones of inhibition, which was measured with the aid of a calibrated ruler<sup>11,13</sup>.

**Statistical analysis:** Data generated were analyzed into tables, charts and figures using simple percentages and descriptive statistics.

## RESULTS

### Distribution of *Salmonella* in samples collected from the teaching and research poultry farm, University of Maiduguri:

The number of positive samples was found to be 19. Highest prevalence rate of 53.33% was obtained for samples from faeces, followed by litters (33.33), whereas, low prevalence rate of 20.00% was recorded for samples obtained from feeds and drinking water, respectively. The overall prevalence of *Salmonella* in poultry samples from University of Maiduguri teaching and research poultry farm was found to be 31.67% (Table 1).

### Distribution of *Salmonella* in samples collected from Wilson Poultry Farm, Fori Ward Maiduguri:

Twenty eight positive samples were obtained. Higher prevalence rate of

80.00% was obtained for samples from litters, followed by faeces (66.67%) whereas, low prevalence rate of 26.67% and 13.3% was obtained for samples from drinking water and feeds, respectively. The overall prevalence of *Salmonella* in poultry samples from Wilson Poultry Farm was found to be 46.67% (Table 2).

**Biochemical characterization of *Salmonella* isolates:** About 44 samples were found to be positive for citrate utilization test, 47 samples each were found to be positive with urease and triple sugar iron tests (TSI) (Table 3).

### Citrate utilization and urease test for *Salmonella* isolates:

Of the total number of positive samples (47) obtained, 44 *Salmonella* isolates were found to utilize citrate with characteristic change in colour from green to blue.

All the 47 positive isolates of *Salmonella* were subjected to urease test were found to be urease positive with characteristic change in colour from amber to pinkish.

### Triple sugar iron test for isolates of *Salmonella*:

All the 47 samples showed the production of hydrogen sulfide (H<sub>2</sub>S), while 23 of the total positive samples of *Salmonella* isolates exhibited gas production. All the positive samples also showed red slope, whereas, 30 of the total positive samples showed a yellow butt. All samples exhibiting these characteristics were found to be *Salmonella*.

### Antimicrobial susceptibility test for *Salmonella* isolates:

The resistance and sensitivity pattern were displayed by 24 *Salmonella* isolates to 10 antimicrobial agents. Septrin (SXT), Amplicin (PN) and Ceporex (CEP) had the highest resistance of 5 (10.64%), 4 (8.51%), 4 (8.51%) to *Salmonella* isolates, followed by Nalidixic Acid (NA)-3 (6.38%),

Table 1: Distribution of *Salmonella* isolates in poultry samples from University of Maiduguri Teaching and Research Poultry Farm, Nigeria

Sampling location	Sample identity	Sample type	Total number collected	Number of positive sample	Prevalence (%)
University of Maiduguri	Fc	Faeces	15	8	53.33
Teaching and Research Poultry Farm	Fd	Feed	15	3	20.00
	Lt	Litter	15	5	33.33
	Dw	Drinking water	15	3	20.00
Total			60	19	31.67

Fc: Faeces, Fd: Feeds, Lt: Litters, Dw: Drinking water

Table 2: Distribution of *Salmonella* isolates in poultry samples from Wilson Poultry Farm Fori Ward Maiduguri, Nigeria

Sampling location	Sample identity	Sample type	Total number collected	Number of positive sample	Prevalence (%)
Wilson Poultry Farm	Fc	Faeces	15	10	66.67
Fori Ward, Maiduguri	Fd	Feed	15	2	13.33
	Lt	Litter	15	12	80.00
	Dw	Drinking water	15	4	26.67
Total			60	28	46.67

Fc: Faeces, Fd: Feeds, Lt: Litters, Dw: Drinking water

Table 3: Biochemical characterization of *Salmonella* isolates from poultry samples in Maiduguri, Nigeria

Sampling location	Sample type	Bacterial growth in broth and on agar		Biochemical examination		
		Selenite F	DCA	Citrate	Urease	TSI
Teaching and Research Poultry Farm University of Maiduguri	Faeces	13	8	8	7	11
	Feeds	5	3	2	2	3
	Litters					
	14	5	6	8	5	
	Drinking Water	9	3	3	4	3
Wilson Poultry Farm	Faeces	9	10	9	10	7
Fori Ward Maiduguri	Feeds	13	2	2	3	2
	Litters	12	12	11	9	12
	Drinking Water	5	4	3	4	4
Total		80	47	44	47	47

DCA: Deoxycholate citrate agar, TSI: Triple sugar iron agar

Table 4: Antimicrobial susceptibility test for *Salmonella* isolates from poultry samples in Maiduguri, Nigeria

Antimicrobial agents	Number of isolates	Resistance (%)	Number of isolates	Intermediate (%)	Number of isolates	Sensitive (%)
Tarivid (OFX)	2	4.26	0	0.00	10	21.28
Reflacine (PEF)	2	4.26	1	2.13	9	19.15
Ciproflox (CPX)	0	0.00	0	0.00	12	25.53
Augmentin (AU)	1	2.13	2	4.26	9	19.15
Gentamicin (CN)	3	6.38	1	2.13	8	17.02
Streptomycin (S)	2	4.26	3	6.38	7	14.89
Ceporex (CEP)	4	8.51	3	6.38	5	10.64
Nalidixic Acid (NA)	3	6.38	3	6.38	6	12.77
Septrin (SXT)	5	10.64	2	4.26	5	10.64
Amplicin (PN)	4	8.51	1	2.13	7	14.89

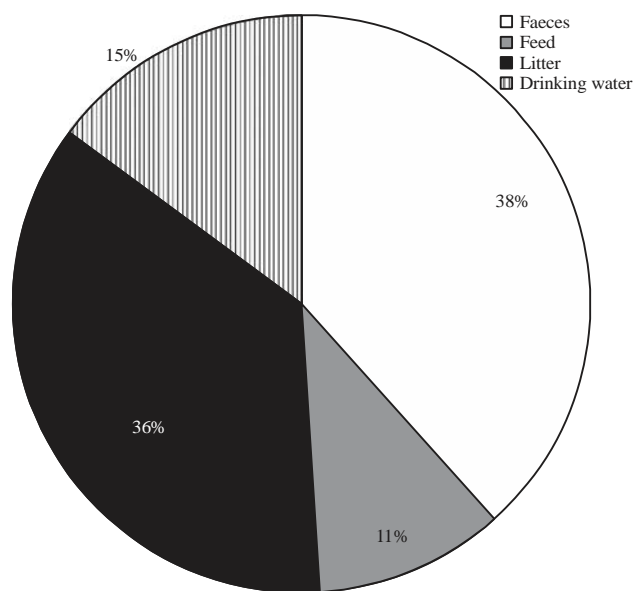


Fig. 1: Distribution of *Salmonella* isolated from two different poultry farms based on sample distribution

Gentamicin (CN)-3 (6.38%), Streptomycin (S)-2 (4.26%), Reflacin (PEF)-2 (4.26%), Tarivid (OFX)-2 (4.26%) and Augmentin (AU)-1 (2.13%), respectively, 0 (0.00%) was found in Ciproflox (CPX). The highest intermediate value were 3 (6.38%) for Nalidixic Acid (NA), Ceporex (CEP) and

Streptomycin (S), followed by 2 (4.26%) for Septrin (SXT) and Augmentin (AU), followed by 1 (2.13%) for Amplicin (PN) and Reflacin (PEF), 0 (0.00%) were found in Ciproflox (CPX) and Tarivid (OFX). The *Salmonella* isolates were sensitive to Ciproflox (CPX), 12 (25.53%), Tarivid (OFX), 10 (21.28%), Augmentin (AU) and Reflacin (PEF), 9 (19.15%), Gentamicin (CN), 8 (17.02%), Amplicin (PN) and Streptomycin (S), 7 (14.89%), Nalidixic Acid (NA), 6 (12.77%), Ceporex (CEP) and Septrin (SXT), 5 (10.64%), respectively (Table 4).

***Salmonella* isolated from two different poultry farms based on sample distribution:** Of the samples collected, the highest prevalence of *Salmonella* recorded was found with the samples obtained from faeces (38%) followed by litters (36%), whereas, drinking water and feed had the lowest prevalence rate of 15% and 11%, respectively (Fig. 1).

## DISCUSSION

*Salmonella* is an enteric pathogen that is shed predominantly in faeces resulting in faecal contamination of feed and water<sup>14,15</sup>. Salmonellosis is a major public health concern and continues to have a serious economic importance in the poultry industry in all countries<sup>16</sup>. With the great expansion of the poultry industry, the wide spread

occurrence of the avian salmonellosis has ranked it as one of the most important egg-borne bacterial diseases of poultry.

The present study was conducted to determine the prevalence and antimicrobial susceptibility of *Salmonella* associated with poultry accessories in Maiduguri, Borno state. Out of the total of 120 samples examined for *Salmonella*, 47 tested positive for *Salmonella*. The overall prevalence of *Salmonella* in this study was found to be 78.33%, which was higher than 43.6% reported in 5 different regions of Nigeria<sup>17</sup>. Furthermore, the prevalence of 30.00% was recorded for faecal samples, followed by 28.33% for litters, while drinking water and feeds recorded 11.67 and 8.33%, respectively. The prevalence reported in this study is higher than those documented for laying hens in EU countries, with overall prevalence of zoonotic *Salmonella* serovars of 2.5%. The high prevalence observed in this study may be attributed to lack of implementation of control programmes on poultry farms and differences in terms of *Salmonella* status among countries but could be influenced by housing system, local environmental conditions, sample types, collection seasons, laying period, isolation methodologies and culture media.

The highest *Salmonella* prevalence per type of poultry sample found in faeces is 30.00%, which was closely followed by litters, 28.33%, then drinking water and feeds had the prevalence of 11.67 and 8.33%, respectively. The 30.00% prevalence for faecal samples found in this study is higher than 11% reported in chicken faeces from Ibadan<sup>18</sup>. The 28.33% prevalence of *Salmonella* associated with litters is higher than 15.63% earlier reported<sup>19</sup>. On the contrary, the prevalence reported for feed and drinking water in this present study were lower when compared with previous studies<sup>19</sup>. Moreover, the rate of *Salmonella* isolation from feed and water samples could be an indication of poor sanitation, handling and contamination along the poultry production chain as well as cross contamination, which calls for serious concerns. Although water was the least contaminated of the four samples, it is of particular concern since birds are exposed to microbial load whenever they drink water.

In most cases, unhygienic packaging could serve as a source of contamination in feed. Contamination may also occur during processing, transport and distribution of compounded feed mixture. The prevalence of *Salmonella* in feed appeared to be quite low. *Salmonella* contamination is not uniformly distributed in the feed and to detect the level of contamination accurately, several hundred samples are required<sup>20</sup>.

Further investigations could be useful to clarify the sources of infections and factors leading to the widespread isolation of *Salmonella* in Nigerian poultry farms. Antimicrobial resistance was determined for ten commonly used agents on fifteen *Salmonella* isolates. In this study, 29.16% resistance to Augmentin has been reported, which is lower than 100% resistance of *Salmonella* from poultry sources<sup>21</sup>.

Of the total samples subjected to antimicrobial susceptibility test, resistance to Nalidixic acid was 41.66%, Gentamicin 33.33% and Augmentin was 29.16%. Sensitivity to Ciproflox was found to be 100%, Tarivid, 41.66% Reflacin 29.16% and Streptomycin and Ampicillin were 25.00%. The development of quinolone-resistant *Salmonella* strains, especially those of poultry meat origin which is gradually leading to an epidemic<sup>22-24</sup>. Nalidixic acid is able to develop resistance quite rapidly. Several studies have also shown that resistance to Nalidixic acid has been on the increase.

## CONCLUSION

The overall prevalence of *Salmonella* was 78.33% obtained from faeces, feeds, litters and drinking water. This study has shown high sensitivity to Ciproflox (100%) and Tarivid (41.66%), whereas high resistance was recorded for Nalidixic acid (41.66%), Gentamicin (33.33%) and Augmentin (29.16%). The relatively high prevalence could be linked to poor infrastructure and low biosecurity measures on poultry farms in the study area and can form the basis for instituting effective intervention strategies.

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