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# Poultry Environment and farm Practices Influencing the Isolation rate of Multi-Drug Resistant *Salmonella* from water and Poultry feed in Zaria, Nigeria

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**ABSTRACT**

*Salmonella* is still a global concern of the food and livestock industries. We evaluated environmental influences and farm practices on rate of *Salmonella* feed and water contamination in selected commercial poultry farms in Zaria. A total of 188 feed samples were collected from randomly selected feed sales outlets and poultry farms using sterile polyethene bags. 94 water samples from primary water sources, reservoirs and drinkers in the poultry farms were collected using sterile universal bottles. Samples were cultured using selective isolation method with prior enrichment. Suspected isolates were identified and characterized using conventional biochemical methods. Eight each of water and feed samples were positive for *Salmonella*. Husbandry systems, hygienic practices, presence of rodents and other environmental factors on the isolation rates of *Salmonella* from samples were correlated. All *Salmonella* isolates were from flocks on deep litter, three *Salmonella* isolates were from commercial and five from self milled feeds on-farm. Isolated *Salmonella* organisms showed highest susceptibility to ciprofloxacin but resistant to commonly used antibiotics. Feed, water, rodents and unhygienic practices are important means of multi-drug resistant *Salmonella* dissemination; they may also serve as critical control points for *Salmonella* in to poultry flocks.

## 1. INTRODUCTION

In many developing countries, chickens represent a major source of animal protein. Family poultry makes up nearly 80 % of all the poultry products in the developing nations. Therefore, efforts in increasing the quality and productivity of backyard chicken will thus provide an immediate impact on the quality of life of the rural poor [1]. In recent years, the poultry industry has expanded in most developing nations with a concomitant requirement for trade in hatching eggs, day old chicks, feed additives and feed concentrates from various controlled and uncontrolled local and international sources [1]. Aside vertical transmission, prominent amongst other sources of *Salmonella* infections into poultry include contaminated feed and feed ingredients, water, equipments, personnel, rodents and hatchery related unhygienic activities [1, 3, 4,5]. Animal-derived protein sources and oil seed meals have long been established as major sources of risk among feed materials, through which *Salmonella* may be introduced to industrial compound feed and feed mills [3, 6]. It is based on this that international regulations require that food and feed are free from *Salmonella*.

Therefore, appropriate process control and decontamination procedures must be adhered to during feed processing to reduce the contamination of feedstuffs and avoid the dissemination of contaminated feed to livestock. Researchers have shown that animals can become infected as a result of consuming *Salmonella* contaminated feed, some of these animals may show clinical disease or carry *Salmonella* without showing any signs. It is also highly possible for *Salmonella* to be transmitted from these animals to food products derived from such animals [6]. It is therefore important to check all raw materials, especially cereals and protein sources, for *Salmonella* contamination. Rodents destructive roles on infrastructures, feed and feed ingredients are well known to farmers but their role as especially multi-drug resistant *Salmonella* reservoir have been underestimated [6]. Major challenges to tropical poultry production include quality feed, flock health and environmental control [6, 7, 8, 9,]. Antibiotic resistance by microorganisms especially *Salmonella* is a global issue [9, 10, 11, 12], as multi-drug resistant *Salmonella* had developed in recent years to which no antibiotic appears to completely eliminate *Salmonella* infections in flocks [9, 10, 13]. Fowl typhoid, is caused by *Salmonella* Gallinarum which is an avian-specific pathogen. Though often underreported, it accounts for about 10% chicken

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mortality in the developing world [3, 8].This study was therefore designed with the following objectives in mind: To better understand poultry *Salmonella* environmental sources/reservoir and patterns of distribution to enable significant improvement in their control strategies. To further determine possible roles of farm hygienic practices on isolation rate of *Salmonella* organisms. Finally, to determine the level of antimicrobial susceptibility to commonly used antibiotics in the study area.

## 2. MATERIALS AND METHODS

### 2.1 Study area

The study was conducted in Zaria, Kaduna state, Nigeria with agro-climatic conditions typical of savanna vegetation located between longitude 11°07N and latitude 7°44E. The poultry industry in this area like in other parts of the country is very fast developing but dominated by sector III (FAO classification) poultry farms [12]. Important water sources to poultry farms include wells, public boreholes and tabs, and harvested rain water.

### 2.2 Sampling procedure

A total of 94 feed samples were collected (5 each from commercial feed outlets, toll-milling stands and self-milled feeds and 79 feed samples cutting across the 3 sources but collected from feeders in poultry houses) between the months of December 2010 to July 2011. The commercial feed brands were vital (VF) Hybrid (HF), Livestock (LF), Rebson (RF), Top (TF) and PLS feeds which included grower, layer, finisher, starter and chick mash. 10 grams of feed was collected midway into the feed bag/feeder using sterile universal bottles. 100 ml of water was collected directly from primary source (bore hole, well and tap) and from secondary sources (reservoirs and drinkers) transported to the laboratory and processed within 2 hours of collection.

### 2.3 Isolation and identification of *Salmonella*

To each 10g of feed type was added 90 ml of sterile one broth *Salmonella* for enrichment in a stomacher and thoroughly mixed for 1 min. The homogenates was then poured into sterile conical flask and incubated at 37 °C for 24 h. a loopful of the thoroughly shaken homogenates was streaked on XLD plate to ensure isolated colonies which was then incubated 37°C for 24 h. Colonies appearing pinkish with or without black centers on XLD were picked and inoculated into Triple Sugar Iron (TSI) agar and Urea agar.

Colonies that gave reactions suggestive of *Salmonella* i.e. alkaline/acid with or without gas and hydrogen sulphide on the TSI, urease negative were kept at 4°C on Nutrient agar (NA) slants until further characterization [13, 14].

### 2.4 Biochemical characterization of isolates

This was done based on standard techniques in which all isolates that gave reactions typical of *Salmonella* were considered to belong to the genus *Salmonella*. The reactions typical of *Salmonella* were indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, motile in motility medium, produce $H_2S$, nitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, manitol, ducitol, and maltose but fail to ferment lactose, sucrose, adonitol and raffinose [13, 14].

### 2.5 Evaluation of the *in-vitro* susceptibility of the isolates to antimicrobial agents

All the biochemically confirmed *Salmonella* isolates were tested for anti-microbial susceptibility to 8 antimicrobial agents with the following disc contents: Chloramphenicol, CH (30 µg), Gentamycin, GN (10 µg), Norfloxacin, NO (10 µg), Ciprofloxacin, CP (10 µg), Tetracycline TE (30 µg), Amoxicillin clavulanate, AU (30 µg), Ampicillin, AM (30 µg), Nalidixic acid, NA and Nitrofurantoin, NF (30 µg), by the disc diffusion method described by Bauer, Kirby, and Turck [14] and based on recommendations of CLSI [13, 14]. The outcome of the susceptibility testing was qualitatively recorded as sensitive or resistant.

## 3. STATISTICAL ANALYSIS

All data collected were analysed for incidence of isolation rate and their antimicrobial susceptibility profile using simple descriptive statistics.

## 4. RESULTS

### 4.1 Microbial analysis

Of the 188 feed and water samples processed, 51 (27%) *Salmonella* suspects were obtained (34 (18%) from feed samples and 17 (9%) were from water samples) and subjected to further biochemical tests. All the biochemical reactions were noted and each suspect was classified based on its biochemical reaction. Of the 51 suspects, 8 (15.7%) (4 each from feed and water) isolates were confirmed to be *Salmonella* from farms B, C, D, M. Samples of commercial poultry feeds gotten from retailer shops were not positive for *Salmonella*. 4(11.76%) isolates showed typical of *Salmonella* appearance from the 34 suspected feed samples. The 17 suspected water samples yielded 4(23.53%) isolates that showed typical *Salmonella* appearance. The remaining suspects were unclassified.

### 4.2 *In vitro* susceptibilities of the *Salmonella* isolates to 8 antimicrobial agents

All the 8 positive *Salmonella* subjected to disc diffusion method showed high sensitivity to ciprofloxacin as indicated by the greatest diameter of the zone of inhibition followed by Gentamicin. However, Norfloxacin, Tetracycline, Amoxicillin, Ampicillin, Nitrofurantoin, and Chloramphenicol, were all found to be resistant.

### 4.3 Environmental and farm practices influencing the isolation rates of *Salmonella*

The influence of routine farm management practices on the occurrence of *Salmonella* was determined using the information obtained from structured questionnaires, and each factor obtained

was correlated with the incidence of *Salmonella* isolates. All the 8 isolates were found in houses that raised birds on deep litter system (Table 1). Flock sizes of between 250-500 birds had the highest isolation rate of 4% , while flock sizes of less than 250 and greater than 500 had isolation rates of 2% each (Table 1). 5% isolation rate was found in houses that used mash type of feed while 3% isolation rate was seen in houses that used the pelletized type feed (Table 1).

**Table. 1:** Distribution of *Salmonella* isolates based on management system, feed and water handling.

| | ***Salmonella* positive** | ***Salmonella* negative** | **Total** |
|---|---|---|---|
| Management system | | | |
| Deep litter | 8 | 86 | 94 |
| Battery cage | 0 | 0 | 0 |
| **Nature of feed** | | | |
| Pelletized | 3 (37%) | 31 (33%) | 34 |
| Mash | 5 (63%) | 55 (58.5%) | 60 |
| **Flock size** | | | |
| Less than 250 | 2(25%) | 17(18.09%) | 19 |
| 250-500 | 4(50%) | 44(46.81%) | 48 |
| Greater than 500 | 2(25%) | 25(26.60%) | 27 |
| **Water source** | | | |
| Borehole | 3 (38%) | 33(35.11%) | 36 |
| Well | 4 (50%) | 27 (28.72%) | 48 |
| Pipe-borne | 1 (13%) | 26 (27.66%) | 27 |
| **Water storage** | | | |
| Reserved | 5(63%) | 47(50.00%) | 52(55.32%) |
| Not reserved | 3(38%) | 39(41.49%) | 42(44.68%) |
| **Water treatment** | | | |
| Treated | 1 (13%) | 27(28.72%) | 28 |
| Not treated | 7 (88%) | 59(62.77%) | 66 |

**Table. 2:** Distribution of *Salmonella* isolates based on some basic biosecurity measures in place.

| **Protective clothing use** | ***Salmonella* positive (+)** | ***Salmonella* negative (-)** | **Total** |
|---|---|---|---|
| Yes | 2(25%) | 25(26.60%) | 27(28.72%) |
| No | 6(75%) | 61(64.89%) | 67(71.28%) |
| **Foot bath** | | | |
| Available | 1 (13%) | 34(36.17%) | 35 |
| Not available | 7 (88%) | 52(55.32%) | 59 |
| **Presence of rodents** | | | |
| Present | 7(88%) | 73(77.66%) | 80(85.11%) |
| Absent | 1(13%) | 13(13.83%) | 14(14.89%) |
| **Presence of fence** | | | |
| Present | 2(25%) | 61(64.89%) | 63(67.02%) |
| Absent | 6(75%) | 25(26.60%) | 31(32.98%) |
| | 8 | 86 | 94 |

Based on source of poultry drinking water, 3% *Salmonella* rate of isolation was found in houses using borehole water, 4% isolates were found in houses using well water and 2% isolates were found in houses using pipe-borne water (Table 1). 5% *Salmonella* isolation rate was for poultry houses that reserved water before use, while 3% isolation rate was recorded in houses that did not reserve water before use (Table 1). Houses that never treated poultry drinking water before use had the highest isolation rate of 7%, while only 1% isolate was found in houses that regularly treated poultry drinking water before use (Table 1). *Salmonella* isolation rate of 6% was recorded in poultry farms that never used protective clothing; however, 2% isolation rate was seen in poultry farms that used protective clothing (Table 2). Houses where foot bath was not being used gave 7% *Salmonella* isolation rate but only 1% isolation rate was seen in houses that foot bath was functional (Table 2).

Isolation rate of 7% was established in poultry farms that had rodents in their farm premises while farms free of rodents had 1% isolation rate (Table 2). Poultry farms that were fenced had 2% *Salmonella* isolation rate in comparison to 6% *Salmonella* isolation from farms that were not fenced (Table 2).

## 5. DISCUSSIONS

*Salmonella* is an enteric pathogen that is shed predominantly in faeces making faecal pollution the main source of feed and water contamination [7, 10] Therefore, the deep litter system of poultry management becomes a leader in the sustenance and transmission of *Salmonella*. Little or no attention has been given to farm management practices and rodent control in poultry farms. It is obvious from this study that farm management practices ranging from choice of production system, stocking density, routine hygienic practices and rodent control had significant influences on *Salmonella* persistence on farms.

Table 1 further showed that all types of feed (pelletized or mash) could be contaminated, with a higher incidence in mash type feed. However, it is on record that heat treatment of feed (as done in pelletization process) is a common means of feed sanitation. In this study it is clear that heat treatment does not protect feed against recontamination during transportation and storage. From this study it may be deduced that bacterial contamination of feed occurred since all the *Salmonella* isolates were from on-farm feeds and none from commercial or toll mill feeds at their various outlets. We believe as recommended that a multiple strategy encompassing heat and antimicrobial treatments with organic acids is required for the reduction of bacterial burden and improvement of feed hygiene [4, 5]

It is alarming to observe all sources of drinking water to poultry which were also same sources to many humans in the study area to contain multidrug resistant *Salmonella*. It is an established fact that antimicrobial resistant bacteria or antimicrobial resistance genes can be transmitted via feed or water [2, 4, 10]. In fact *Salmonella* can persist and grow in water given the right conditions and that the diversity and concentration of *Salmonella* increases as temperatures rises. Therefore, a better approach to *Salmonella* control in farms will also involve the microbiological test of water especially if the source of water is a well or river [6, 7, 15]. It is on record and as seen in this study that contamination of the farm environment can be a source of *Salmonella* infection, and that improving farm environment and personnel hygiene had decreased *Salmonella* prevalence [2, 5, 6]. Water and feed acidification had minimized *Salmonella* infection and promoted good gut health, thereby enhancing the animal productivity [1, 2, 4, 5]. This study supports the fact that rodents for decades have been known for their role as reservoir of *Salmonella* organisms that can contaminate feed, water and

environment and transmit organisms to poultry [6, 11]. The public health concern of this study stems from the fact that around 2.6%, 10.6% and 17.0% of human salmonellosis cases are attributable to turkeys, broilers and laying hens, respectively [4,6,15]. Further, the result of the *Salmonella* anti-microbial resistance profile in this study has two major concerns; first the isolates are multi-drug resistant implying commonly used, cheap and readily available antibiotics in the study area will not be effective against salmonellosis of both poultry and probably humans. Secondly, norfloxacin resistance is of concern because it belongs to the fluro-quinolones which constitute drug of choice of human and poultry salmonellosis. This may be responsible for salmonellosis that is refractory to treatment in both human and poultry.

## 6. CONCLUSION

Timely identification of *Salmonella* from clinical samples, contaminated feed or water prevents *Salmonella* spread within flocks and possible entry in to food chain. There is therefore the need to institute *Salmonella* monitoring in poultry farms to reduce incidence of poultry and human salmonellosis.

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