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# PREVALENCE AND ANTIBIOGRAM OF *Salmonella* spp FROM POULTRY FEEDS AND FECAL DROPPINGS OBTAINED FROM SELECTED FARMS IN UMUDIKE, UMUAHIA

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## ABSTRACT

*Salmonella species are among the leading causes of foodborne gastrointestinal diseases, frequently transmitted through poultry products. This study investigated the microbial load, prevalence, and antimicrobial resistance profiles of Salmonella spp from poultry sources. A total of 144 samples, comprising 72 poultry feed samples and 72 fecal dropping samples, were analyzed using standard culturing techniques on bacteriological media. Salmonella isolates were identified through cultural and biochemical tests, followed by antimicrobial susceptibility testing via the Kirby–Bauer disc diffusion method. Results revealed that fecal droppings exhibited significantly higher bacterial loads, with total viable counts (TVC) ranging from 92.6 to 129.3 x* $10^5$ *CFU/g, compared to poultry feeds, which ranged from 14.9 to 45.4 x* $10^5$ *CFU/g. Increased prevalence of Salmonella spp. was observed in fecal droppings (48.6%) compared to poultry feeds, with the highest prevalence recorded in feed samples given to growers (66.7%) and the lowest in feed samples for starters (0.0%). Antimicrobial resistance testing demonstrated that Salmonella isolates showed high resistance to erythromycin, augmentin, and ampicillin, while exhibiting sensitivity to gentamicin, ciprofloxacin, and ofloxacin. The findings highlight the role of poultry feeds and fecal droppings as major reservoirs of Salmonella spp., posing a potential risk of food poisoning without proper hygienic practices during poultry rearing, handling, and processing.*

**KEYWORDS:** *Salmonella, Fecal droppings, Poultry feed, Antimicrobial Resistance*

## INTRODUCTION

The poultry industry plays a crucial role in supporting the economy of any nation. However, it is frequently disrupted by disease outbreaks, leading to significant losses in both chicken and egg production. While poultry and poultry products are major contributors to the food chain, providing affordable, high-quality protein (122.5 million tons per year they are also recognized as key reservoirs of foodborne pathogens (OECD/FAO, 2020). The presence of these harmful microbial contaminants in livestock feed poses a serious food safety risk, threatening both animal and human health. The microorganisms primarily involved are foodborne pathogens, with *Salmonella* and *Campylobacter* spp. being the most common causes of human foodborne bacterial illnesses linked to poultry (Okike and Ugwu, 2021; Mgbeahuruike *et al*., 2023).

The contamination of poultry feed with microorganisms, has three major consequences: firstly a decrease in the feed's composition and quality, which can lead to poor performance, secondly, the production of toxins that may impair performance or affect health, depending on the type and dosage of toxins, and thirdly, the colonization of the animal's gut by the microbes, which can lead to complex effects, ranging from beneficial impacts like gut colonization and microbiota development, to negative effects such as reduced feed efficiency. These adverse outcomes can result in financial losses for farmers due to poor feed conversion, slower growth rates or weight gain, damage to organs and tissues, changes in sensory qualities, reduced egg production, lower quality eggs and meat, and even increased mortality rates (Ezekiel *et al*., 2012). Also, recent studies have shown that an organism derived from contaminated poultry feed, specifically *Bacillus cereus*, can worsen viral diseases such as avian influenza in poultry (Zhang *et al*., 2019). While viruses cannot replicate outside of host cells, they can infect poultry when the birds consume feed contaminated with droplets from infected individuals, whether human or animal (Serbessa and Tucho, 2017).

Salmonellosis is a significant foodborne disease in the poultry industry, transmitted through meat, meat products, eggs, and egg products (Madaki *et al.,* 2019). This contamination typically occurs either through direct animal infection or fecal contamination during processing (Hossain *et al.,* 2011). According to the U.S. Centre for Disease Control and Prevention, poultry-related Salmonella infections account for more than 50% of human Salmonella outbreaks (Roth, 2012). In Nigeria, where poultry product consumption is high, there has been a noticeable increase in cases of Salmonella-associated gastroenteritis (Fasure *et al*., 2012). Many feed concentrates, particularly those containing fish or meat meal, can be potential sources of *Salmonella* spp., serving as a route for infection in intensively reared poultry.

Contaminated livestock feed can also serve as a source of antimicrobial-resistant (AMR) organisms, making AMR in livestock feed a significant food safety concern. The intensification of poultry farming is a key driver of increased antimicrobial use (Van Boeckel *et al*., 2015). In feed mills and integrated feed manufacturing companies, poultry feed is often supplemented with antibiotics (feed additives), sometimes including last-line antibiotics like colistin, for prophylactic control of microbial (especially bacterial and protozoan) infections and to promote growth (Chattopadhyay, 2014; Manikandan *et al*., 2020). Premixes, which are combinations of inorganic minerals and vitamins used in poultry feed, can also contain antimicrobial agents. Consequently, individuals handling these feeds are at high risk of exposure to resistant organisms, which could then spread to the broader population. Therefore, it is essential to understand the prevalence and impact of microbial contaminants in poultry feed. Also, information about the variety of microbial contaminants, and their potential public health consequences is crucial for developing effective feed monitoring and hazard control strategies.

This study was designed to ascertain the microbial load of the poultry products and select the appropriate antibiotics against *Salmonella* determining the resistance and sensitivity patterns of the isolates with the view to make people more prudent in the use of antibiotics. Michael Okpara University of Agriculture and the National Root Crop Research Institute share a large campus in Umudike, Abia State (approx. 5.48°N, 7.55°E). The environment features a hot, humid tropical monsoon climate with very high rainfall. Both institutions are located off the Umuahia-Ikot Ekpene Road, approximately 10-15 km southeast of Umuahia, the capital of Abia State.

## MATERIALS AND METHODS

### *Sample Collection and Sample Sizes*

A total of 144 samples were collected from feed and fecal dropping from two selected poultry farms in Umudike; Michael Okpara University of Agriculture, Umudike (MOUAU) poultry farm and National Root Crop Research Institute (NRCRI) poultry farm. 72 feed samples were collected from three (3) different kinds of feed, vital feed, Amobng feed and Top feed. The remaining 72 samples were collected from fecal droppings of the poultry birds fed with the different feed samples. The distribution is shown in table 1.

Sterile bags were used to collect the feed samples from the poultry farms to the Microbiology laboratory for isolation and identification of *Salmonella* while 10 grams of fresh fecal dropping samples were collected from poultry birds aseptically by using a sterile cotton bud. All the samples were transported immediately to the laboratory to avoid contamination.

Table 1: Summary of Samples Collected According to Sources and Locations

| Sample type | Location | Feed type | No of samples | Total |
|---|---|---|---|---|
| Amobyng Feed | MOUAU P. farm | Starter | 6 | 18 |
| | | Grower | 6 | |
| | | Layer | 6 | |
| Fecal dropping | MOUAU P. farm | | 18 | 18 |
| Top feed | NRCRI P. farm | Starter | 6 | 18 |
| | | Grower | 6 | |
| | | Layer | 6 | |
| Vital feed | NRCRI P. farm | Starter | 6 | 18 |
| | | Grower | 6 | |
| | | Layer | 6 | |
| Amobng feed | NRCRI P. farm | Starter | 6 | 18 |
| | | Grower | 6 | |
| | | Layer | 6 | |
| Fecal dropping | NRCRI P. farm | | 54 | 54 |
| Total Samples | | | | 144 |

### *Determination of Microbial Load*

One gram of the feed sample was homogenized in 9 mL of sterile deionized water, creating a 1:10 dilution. A fivefold serial dilution was then prepared. From each dilution, a 0.1 mL aliquot was transferred onto nutrient agar (Oxoid) using the spread plate technique. The plates were incubated at 37 °C for 18–24 hours. Colony enumeration was performed using a colony counter, and the total bacterial count was calculated using the formula; CFU/g = level of Dilution plated x number of colonies counted/ amount plated (Nkere *et al*., 2011). Bacterial counts below 30 and above 300 were excluded since they were not within the statistically proven range of colonies to be considered when determining total count of bacteria.

### *Isolation of Salmonella from Poultry Feeds*

The feed samples were prepared by suspending 1g of feed sample into a test tube containing normal saline and then 2-3 loopful of the feed suspensions were streaked on a freshly prepared *Salmonella-Shigella* agar and incubated at 37°C for 24hrs. The growths observed were sub-cultured in nutrient agar, then the identification of the isolated organisms was done by Gram staining and biochemical tests.

### *Isolation of Salmonella from poultry fecal droppings*

The fecal dropping samples were prepared by suspending the cotton buds into normal saline in a tube and then 2-3 loopful of the fecal suspensions were streaked on a Salmonella Shigella agar and incubated at 37°C for 24hrs. The growths observed were sub-cultured in nutrient agar and the identification of the isolated organisms was done by Gram-staining and also by biochemical tests.

### *Biochemical Characterization*

All suspected colonies were subjected to various biochemical tests including gram staining, catalase test, oxidase test, methyl-red test and citrate test.

### *Antibiogram Study of the Salmonella Isolates*

All confirmed isolates were subjected to *in vitro* antibiotic susceptibility testing to 7 antibiotics of different classes Disk diffusion method of Bauer and Kirby was implemented following the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2020). The following antibiotics were used in the study; Gentamicin, Ofloxacin, Ampicillin, Ciprofloxacin, Streptomycin, Erythromycin and Augmentin.

## RESULTS

The result of the Microbial load for all samples is presented in Tables 2 and 3. The analysis of variance (ANOVA) revealed significant differences in AMO (M) and VITAL (N) across poultry groups, with Layers feed showing superior microbial counts. Layers feed had the highest mean (45.4 ± 2.79), followed by Growers (35.4 ± 4.14) and Starters (14.9 ± 3.70). However, no significant differences existed between TOP (N) and AMOBYNG (N).

Table 2: Microbial Load of Different feed types from both locations

| SAMPLES | TOP(N) | AMOBYNG(N) | VITAL(N) | AMO(M) |
|---|---|---|---|---|
| **Growers (n=6)** | $16.0^{b}$ ±4.22 | $27.4^{b}$ ± 4.04 | $19.8^{b}$ ± 7.20 | $35.4^{b}$ ± 4.14 |
| **Layers (n=6)** | $17.2^{b}$ ±5.84 | $30.2^{a}$ ± 7.54 | $26.2^{a}$ ± 3.52 | $45.4^{a}$ ± 2.79 |
| **Starter (n=6)** | $20.5^{a}$ ± 5.61 | $28.1^{b}$ ± 10.3 | $15.6^{c}$ ± 5.56 | $14.9^{c}$ ± 3.70 |

Values show means of six replicates ± standard deviation. Values with different superscripts in the same column are significantly different ($p<0.05$). M= MOUAU, N=NRCRI

Table 3: Microbial Load of Fecal droppings from both Locations

| SAMPLES | NRCRI | MOUAU |
|---|---|---|
| **Growers** | $114.6^{a} \pm 5.22$ | $129.3^{a} \pm 7.04$ |
| **Layers** | $112.8^{a} \pm 11.8$ | $121.7^{b} \pm 15.2$ |
| **Starter** | $92.6^{b} \pm 9.27$ | $105.8^{c} \pm 5.03$ |

Values show means of six replicates ± standard deviation. Values with different superscripts in the same column are significantly different p<0.05).

### *The Prevalence of Salmonella* **spp** *in the Various Feed Samples in NRCRI and MOUAU Poultry Farms*

The occurrence of *Salmonella* spp in the various kinds of feeds from both NRCRI poultry farm and MOUAU poultry farm is represented in Table 4. This table showed that the feed samples for growers had the highest prevalence rate of 66.7% *Salmonella* spp while the feed for starters had the lowest prevalence rate.

Table 4: Prevalence of *Salmonella* spp in the various feed samples in NRCRI and MOUAU poultry farms

| Feed Type/Site | Starter (%) | Grower (%) | Layers (%) |
|---|---|---|---|
| **Amobyng (N)** | 0 | 66.7 | 16.7 |
| **Top (N)** | 33.3 | 33.3 | 16.7 |
| **Vital (N)** | 33.3 | 16.7 | 33.3 |
| **Amobyng (M)** | 16.7 | 33.3 | 33.3 |

N= National root crop research institute poultry farm
M= Michael Okpara University of Agriculture poultry farm

### *The Prevalence of Salmonella* **spp** *in Fecal Samples of Poultry Birds in NRCRI and MOUAU Poultry Farms*

The occurrence of *Salmonella* in the fecal samples from both NRCRI and MOUAU poultry farm is represented in Table 5 and it shows that the prevalent rate of *Salmonella* in the fecal droppings were found to be highest in the layers and lowest in the fecal droppings of starters.

Table 5: Prevalence of *Salmonella* spp in fecal samples of poultry birds in NRCRI and MOUAU poultry farms

| Site | No of *Salmonella* species Isolated | % Isolated |
|---|---|---|
| **NRCRI (n= 54)** | 29 | 53.7 |
| **MOUAU (n= 18)** | 6 | 33.3 |
| **Total (n=72)** | 35 | 48.6 |

### *Antibiogram Study*

Antibiogram studies of the *Salmonella* isolates were determined by the observation of 7 common antimicrobial agents against the isolates. These antimicrobial agents include; gentamicin, ofloxacin, ampicillin, ciprofloxacin, streptomycin, erythromycin and augmentin. The antibiogram study of all the *Salmonella* isolates from all the poultry feed and fecal dropping samples showed that gentamicin, ciprofloxacin and ofloxacin had the highest sensitivity rates against the isolates while the isolates were most resistant to augmentin and ampicillin. This is represented in fig 1 to fig 4.

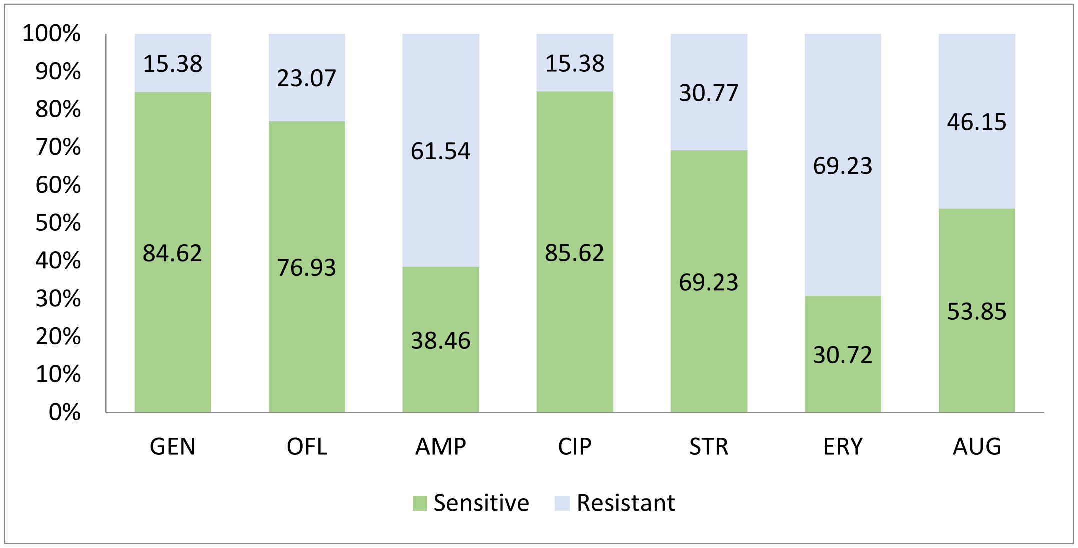

Fig. 1: Antibiotic Sensitivity of *Salmonella* isolates from Amobyng poultry feeds and droppings collected from MOUAU.
Gen= Gentamycin; OFL= Ofloxacin; AMP = Ampicillin; CIP= Ciprofloxacin; STR= Streptomycin ERY= Erythromycin AUG = Augmentin

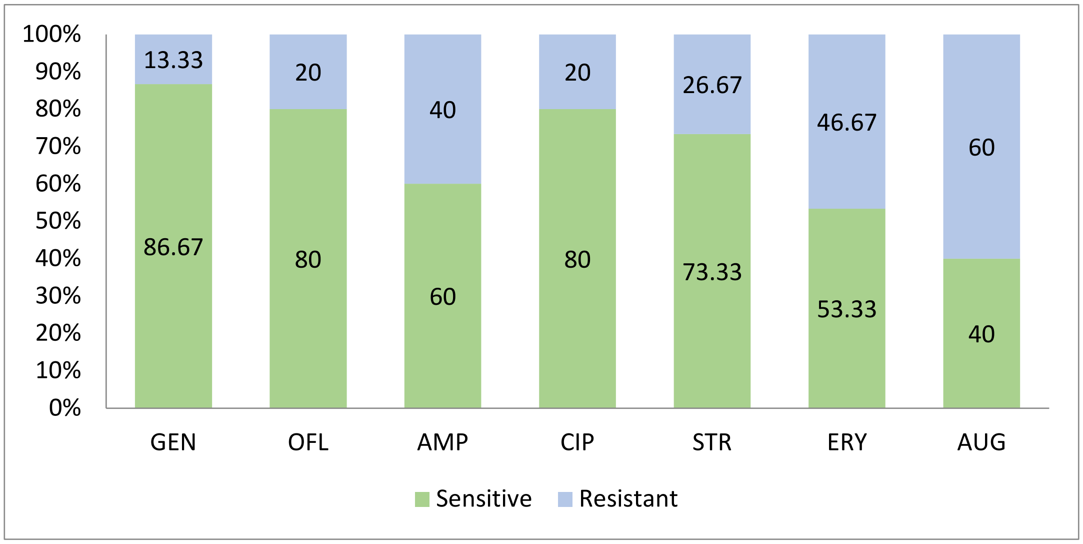

Fig. 2: Antibiotic Sensitivity of *Salmonella* isolates from Vital poultry feeds and droppings collected from NRCRI.
Gen= Gentamycin; OFL= Ofloxacin; AMP = Ampicillin; CIP= Ciprofloxacin; STR= Streptomycin ERY= Erythromycin; AUG = Augmentin

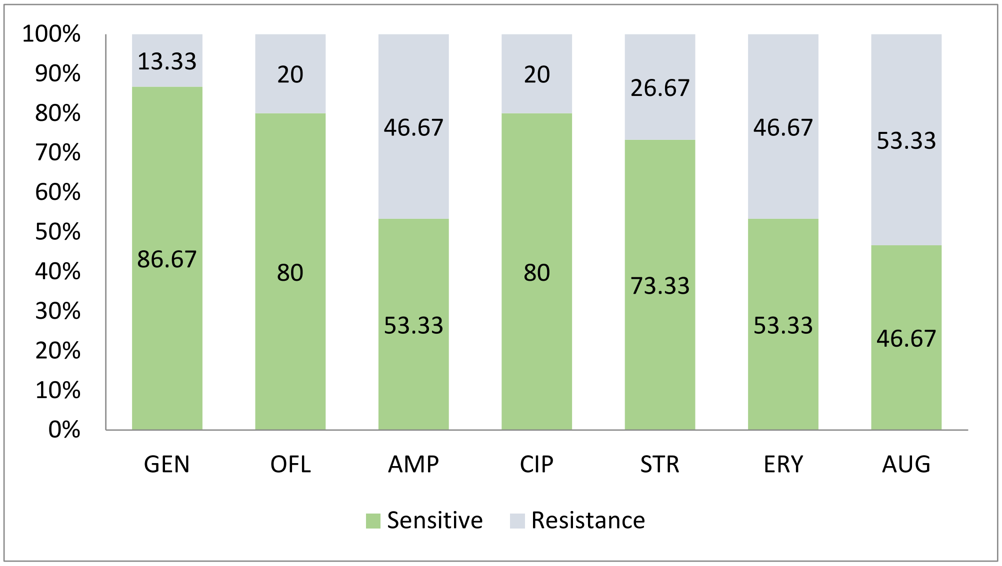

Fig. 3: Antibiotic Sensitivity of *Salmonella* isolates from Top poultry feeds and droppings collected from NRCRI.
Gen= Gentamycin; OFL= Ofloxacin; AMP = Ampicillin; CIP= Ciprofloxacin; STR= Streptomycin ERY= Erythromycin; AUG = Augmentin

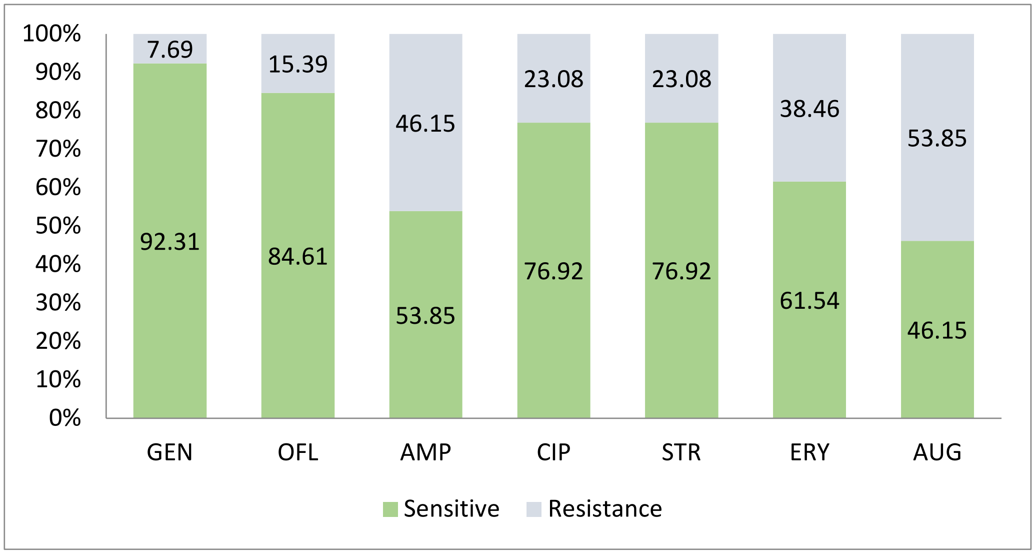

Fig. 4: Antibiotic Sensitivity of *Salmonella* isolates from Amobyng poultry feeds and droppings collected from NRCRI.
Gen= Gentamycin; OFL= Ofloxacin; AMP = Ampicillin; CIP= Ciprofloxacin; STR= Streptomycin ERY= Erythromycin; AUG = Augmentin

## DISCUSSION

This study showed increased prevalence of *Salmonella* occurred in the poultry fecal droppings when compared to the poultry feeds. The highest number of *Salmonella* spp was isolated in Amobyng feed samples given to growers (66.7%) while the lowest number occurred in the same feed samples for starters (0.0%). The study recorded high differences of *Salmonella* occurrence among different feed samples while an overall prevalence of 48.6% of *Salmonella* species were obtained from the fecal droppings of the poultry birds. Possible explanations for the isolation of *Salmonella* from the feed types could be attributed to a couple of reasons. First, if the raw materials used in feed production are contaminated with bacteria like *Salmonella*, they can be carried over into the feed. Jones *et al.* (2018) reported that the main ingredients for poultry feeds are animal protein ingredients which are locally processed fish wastes are important causes of *Salmonella* contamination of feeds. Also, the processing methods employed could play a significant role. If the feed isn't properly heat-treated or pelleted, bacteria might not be killed off. Cross-contamination could also occur during processing. Storing the feeds in a humid environment promotes bacterial growth. Handling by the workers without proper hygiene could also have introduced the organism. The 48.6% prevalence rate of *Salmonella* in the fecal droppings from this study is much lower than the 71.84% reported by Akhtar *et al.,* (2010) and also much lower as compared to the prevalence rates reported by Musa *et al.,* (2014). This study recorded differences of prevalence among samples which satisfied the findings of another researcher Irfan *et al.,* (2015).

The total viable counts (TVC) showed that fecal droppings had more bacterial load than the feed samples. The TVC in the poultry feeds were in the range of 14.9 to 45.4 x $10^5$ $cfug^{-1}$ while the TVC in the poultry droppings were within the range of 92.6 to 129.3 x $10^5$ $cfug^{-1}$. Overcrowded, unsanitary farms could contribute to the increased bacterial load. High bacterial contamination in poultry feeds poses risks to bird health, productivity, and food safety.

In this study, all the *Salmonella* isolates were investigated for susceptibility and resistance to 7 commonly used antibiotics; gentamicin, ofloxacin, ampicillin, ciprofloxacin, streptomycin, erythromycin and augmentin. Among the variety of antibiotics tested, the highest resistance was found with erythromycin, followed by augmentin and ampicillin. On the other hand, most of the isolates were highly sensitive to gentamycin, ciprofloxacin and ofloxacin. The antibiotic resistance patterns of the *Salmonella* isolates to erythromycin, ampicillin and augmentin correlated with the results of Lee *et al.,* (2005) who recorded that erythromycin, ampicillin and augmentin had 89.2%, 70.1% and 70% resistance respectively. The sensitivity profiles of gentamycin, ciprofloxacin and ofloxacin in this study correlates with the research works of Khan (2004). A number of reasons may be responsible for the resistance of the isolates as observed in this study. The overlap between antibiotics used in poultry and human medicine may accelerates cross-resistance. Also, resistance genes linked to disinfectant resistance could amplify survival strategies amongst the isolates. The danger here is that resistant *Salmonella* can infect humans via undercooked meat/eggs, complicating future clinical treatment.

## CONCLUSION

Results of the study suggests that poultry feeds and fecal droppings of poultry birds are major reservoirs of *Salmonella* spp and might be a potential cause of food poisoning if proper hygienic measures are not undertaken during rearing, handling and processing of poultry and poultry sources. Also results of the study show that gentamicin, ciprofloxacin and ofloxacin can be used as first line therapies in the treatment of *Salmonella* gastroenteritis from poultry sources.

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