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Susceptibility Profiles of *Salmonella* Isolated from Poultry Droppings, Rinse Water of Contaminated Eggs and Poultry Feeds in Makurdi, Nigeria

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ABSTRACT

Salmonellosis is a common food-borne bacterial zoonotic disease. The emergence of antibiotic resistance among the *Salmonella* globally is a great public health Hazard. This study was undertaken to isolate, identify and study the Antibiogram of *Salmonella* present in fecal materials, fecally stained egg washings, and poultry feeds. Poultry feeds were obtained from retail shops and live birds market in Makurdi, Benue State, Nigeria. A total of 300 samples were collected, and the isolation and identification of *Salmonella* done according to international standard method; non-selective enrichment, selective enrichment, isolation using Buffered Peptone Water (BPW), Selenite-F-broth and Xylose Lysine Deoxycholate (XLD) agar respectively. Positive samples were processed to study the antibiogram pattern by Kirby-Bauer method. Out of 300 samples, a total 83 samples were found to be positive (27.67%) for *Salmonella.* The prevalence of *Salmonella* in egg washings, fecal materials and poultry feeds was 11%, 72% and 0% respectively. Statistical analysis showed that there was no statistical significant difference (P>0.05) on prevalence of the *Salmonella* among isolates from the sample sources and location. The *Salmonella* isolates were resistant to at least two or more antibiotics. Among tested drugs, *Salmonella* isolates was highly resistant to Ceporex (81.93%) followed by Nalidixic Acid (80.72%), Ampicilin (79.52%), Cotrimoxazole (65.06%) and Augmentin (63.86%). However, *Salmonella* isolates were Sensitive to Ofloxacin (63.85%) and Streptomycin (36.14%). From the results of the study, it is concluded that *Salmonella* isolates seen in poultry origin samples which indicate this could be potential vehicle for antibiotic resistant *Salmonella*-food-borne infection to humans. Hence there is need to create awareness among the public, poultry sellers, farmers and local food vendors of Makurdi-Benue state regarding hygienic practices adaptation, Strengthen Biosecurity Measures, Promote Responsible Antibiotic Use and also implementation of preventive measures

KEYWORDS: Antibiogram, *Salmonella*, Poultry, antibiotics

ARTICLE DETAILS

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1.0 INTRODUCTION

The Nigerian poultry sector has expanded rapidly in recent years. Local production currently meets only 30% of the demand for chicken eggs and meat, indicating significant potential for industry growth.

Nigeria has the largest annual egg production and the second-largest chicken population in Africa, with the industry comprising approximately 180 million birds. Of these, 80 million chickens are raised in extensive systems, 60 million in semi-intensive systems, and 40 million in intensive systems. Poultry production in Nigeria yields up to 300 metric tons of meat and 650 metric tons of eggs per year. About 85 million Nigerians are involved in poultry production, many on a small to medium scale. (FAO,2019).

Poultry meat and eggs are important sources of animal protein in many developing countries because they are cheap and widely accepted (Bettridge *et al.*, 2014; Fagbamila *et al.*, 2017). As key elements of the human food chain, poultry products are major reservoirs of intestinal and food-borne pathogens such as Salmonella, making them a primary source of salmonellosis in humans. The majority of human Salmonella infections stem from the consumption of

contaminated poultry, pork, beef, and eggs (Nurudeen et al., 2018).

Salmonella are Gram-negative, small rod-shaped, non-sporing, non-capsulated, aerobic, and facultative anaerobic organisms that belong to the family Enterobacteriaceae (Akinola *et al.,* 2019). This infectious and contagious bacterium can be transmitted to humans, warm-blooded animals, and reptiles, with poultry birds being mostly vulnerable. Salmonella is linked with various infectious diseases such as typhoidal fever and non-typhoid salmonellosis, which globally present significant public health challenges and cause financial losses in livestock (Ezeigbo *et al.,* 2014).

The wide use of antibiotics in veterinary medicine has led to an upsurge in resistant bacterial strains in recent times (Varga *et al*., 2019). Specifically, the repetitive application of antibiotics in domestic animals as well as poultry for prevention and treatment of disease; and growth promotion has contributed to the emergence of antibiotic-resistant bacteria. These resistant bacteria can be transmitted to humans through the food chain or direct contact with infected animals. The use of antimicrobial agents creates selective pressure, favouring the survival of antibiotic-resistant pathogens.

Globally, multi-drug-resistant strains of *Salmonella* are prevalent in both human and animal isolates. Non-pathogenic, multi-drug-resistant Salmonella in the intestines likely serve as significant reservoirs of resistance genes (Aniokette et al., 2016). Multi-drug resistance complicates the management of Salmonella-caused intra- and extra-intestinal infections, resulting in significant illness, death, and increased healthcare costs (Nhung et al., 2017).

Furthermore, the widespread use of antibiotics in both human and veterinary medicine has increased the prevalence of resistant *Salmonella* strains isolated from poultry and poultry environments. Poultry fed antibiotics as growth enhancers can develop antibiotic-resistant isolates (Velasquez et al., 2018). Antimicrobial-resistant strains of Salmonella are now widespread, with most resistant strains of zoonotic origin, acquiring resistance in animal hosts before being transmitted to humans through the food chain (Parbati et al., 2017; Varga et al., 2019).

Therefore, this study aims to isolate Salmonella from poultry eggs and poultry feeds sold in Makurdi and to assess their resistance and sensitivity patterns to common antibiotics.

2.0 MATERIALS AND METHOD

2.1 Sample Collection and Sample size.

A total of 300 samples comprised of 100 fecally stained egg surface washes, 100 faecal materials and 100 poultry feed samples were collected from live poultry markets, farms and poultry feed shops in Markudi-Benue state. Samples were collected from different live birds Markets (Wurukum Market, Modern Market and North Bank Market) and poultry feeds stores in Makurdi town.

Sample of egg shell surface

Samples from Eggs stained with faecal materials were collected from egg shops by immersing the faecal stained faecal egg shell in 5mls of normal saline and egg shell rinse was immediately stored in sterile test tube, labelled and transported to the advanced biological science laboratory, Joseph Sarwuam Tarkaa University Makurdi.

Sample collection from faecal material: About 5gm of fresh faecal samples were collected aseptically into sterile vials with a spatula, labelled and transported to the advanced biological science laboratory, Joseph Sarwuam Tarkaa University Makurdi.

Sample collection from poultry feeds: About 5gm of commercial poultry feeds were collected aseptically into sterile vials with the help of a spoon, labelled and transported to the advanced biological science laboratory, Joseph Sarwuam Tarkaa University Makurdi.

2.2 Isolation and Identification

The isolation and identification of *Salmonella* was done according to international standard method, ISO-6579 (2017), About 1ml of sample was transferred immediately into sterile test tubes containing 9mls of buffered peptone water and incubated at 37ºC overnight. An aliquot of 0.1ml was inoculated into test tubes containing 10mls of Selenite-F-broth and incubated at 37ºC for 24 hours, a loopful of culture from the selective enriched broth were streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated for 24 hours at 37º C. For samples from faecal materials and poultry feeds, about 1.0 g of sample was added to sterile test tubes containing 9mls of buffered peptone water and incubated at 37ºC overnight. An aliquot of 0.1ml was inoculated into test tubes containing 10 mls of Selenite-F-broth and incubated at 37ºC for 24 hours, a loopful of culture from the selective enriched broth were plated onto Xylose Lysine Deoxycholate (XLD) agar and incubated for 24 hours at 37º C.

The cultural characterization of isolated bacterial was done by observing the colonies for shape, size, colour and opacity displayed on XLD agar. Biochemical examinations included Gram Staining and

Indole test (CLSI, 2013).

2.3 Antibiogram

The total of 83 positive isolates were tested for antibacterial drug susceptibility against 10 commonly used antibiotics belonging to different groups by disc diffusion method first described by Kirby-Bauer (CLSI, 2013). Briefly, a sterile wire loop was used to pick two to three colonies of Salmonella from Xylose-lysine-deoxycholate agar plates and emulsified in 3 to 4ml of sterile normal saline and turbidity matched against Mcfarland No. 0.5 turbidity standard. 10ml of Mueller-Hinton agar was dispensed into 50mm disposable

Petri dishes and allowed to solidify at room temperature in sterile Laminar flow hood. Agar plates were inoculated using sterile wire loops. Antimicrobial discs were applied on Mueller Hinton plates with sterile forceps within 15 minutes. Locally isolated *Escherichia coli* were used as control plate and incubated at 37^{O}C for 24 hours, after which plates were examined. The diameters of the zones of inhibition were measured to the nearest millimeter using a meter rule and compared with a zone interpretation chart (as sensitive, intermediate or resistant) (CLSI, 2013)

2.4 Data Analysis

Person's Chi-square ($\chi 2$) test at a significance level of 0.05 was used to measure association between prevalence of *Salmonella* according to sample source and sample location and results considered significant when $P \leq 0.05$.

3.0 RESULTS

A total of 300 samples were collected, consisting of 100 faecal-stained eggshell surface washes, 100 faecal materials, and 100 poultry feed samples from live poultry markets, farms, and poultry feed shops in Makurdi, Benue State. Among these samples, the overall prevalence of Salmonella was 27.67%, with 83 positive cases recorded and tested against 10 commonly used antimicrobials belonging to four different classes of antibiotics revealed different sensitivity and resistant patterns according to different sample sources and location.

Among tested drugs Ceporex (81.93%) was highly resistant by the isolated *Salmonella* followed by Nalidixic Acid (80.72%), Ampicillin (79.52%), Septrin (65.06%) and Augmentin (63.86%). However, positive *Salmonella* isolates were Sensitive to Ofloxacin (63.85%) and Streptomycin (36.14%). All isolated *Salmonella* spp were resistant to two or more antibiotics as shown in table 1.

Within the sample sources faecal material had the highest number of resistant isolates (397), followed by egg washings 77 resistant isolates and the least was commercial poultry feeds with no resistant isolates. Significant difference was observed across the different sample sources in the distribution of resistant, intermediate, and sensitive Salmonella isolates (table 2).

Across the locations Wurukum market recorded the highest number of resistant isolates (267), followed by Modern market with (167) resistant isolates, North bank market (40) resistant isolates and the least vet shops with no resistant, intermediate, or sensitive isolates. The distribution of antibiotic resistance, intermediate resistance, and sensitivity across the locations was observed to be significantly different. (table 3.)

Out of the antibiotic classes tested (table 4.) fluoroquinolones showed the highest resistance with 157 resistant isolates, followed by Penicillins (119 resistant isolates), Cephalosporins with 144 resistant isolates. Sulfonamides had the lowest

resistance count (54 resistant isolates), The resistance, intermediate resistance, and sensitivity across different classes of antibiotics was observed to be significantly different ($p > 0.05$).

Table 1: Antibiotic study for Resistance, intermediate and Sensitivity for different antibiotics used on the Isolates.

Antibiotic disc	Disc Conc./ Potency	Sensitive %	Intermediate %	Resistant%
Ofloxacin (OFX)	10 µg	53 (63.83%)	6 (7.23%)	24 (28.92%)
Peflacine (PEF)	10 µg	29 (34.94%)	14 (16.87%)	40 (48.19%)
Ciprofloxacin (CPX)	10 µg	23 (27.70%)	34 (40.96%)	26 (31.33%)
Augmentin (AU)	30 µg	19 (22.89%)	11 (13.25%)	53 (63.86%)
Gentamicin (CN)	10 µg	27 (32.53%)	10 (12.05%)	46 (55.42%)
Streptomycin (S)	30 µg	30 (36.14%)	23 (27.71%)	30 (36.14%)
Ceporex (CEP)	10 µg	13 (15.66%)	2 (2.40%)	68 (81.93)
Nalidixic Acid (NA)	30 µg	8 (9.64%)	8 (9.64%)	67 (80.72%)
Septrin (SXT)	30 µg	23 (27.70%)	6 (7.23%)	54(65.06%)
Ampicilin (AMP)	30 µg	9 (10.84%)	8 (9.64%)	66 (79.52%)

Table 2. Antibiotic sensitivity result based on sample source (N=830)

Source		Resistant		intermediate	Sensitive
Egg washings		77		15	18
Faecal material	397		107	216	
Commercial poultry feeds	0		0	0	
Total		**477**		**122**	**234**

$\chi^2 = 54.76$; df = 2; P= 5.991

Table 3. Antibiotic sensitivity result based on sample Location (N=830)

Location	Resistant	Intermediate	Sensitiv
Wurukum market	267	74	129
Northbank market	40	14	16
Modern market	167	34	89
Vet shops	0	0	0
Total	**474**	**122**	**234**

$X^2 = 11.72$; df = 3; P= 9.488

Table 4. Antibiotic study based on class of antibiotics used. (N=830)

Antibiotic class	Resistant	Intermediate	Sensitive
Cephalosporins	144	35	70
Flouroquinolones	157	62	113
Penicillins	119	19	28
Sulfonamides	54	6	23
Total	**474**	**122**	**234**

$\chi^2 = 48.07$; df = 3; P. = 15.507

4.0 DISCUSSION

Antibiogram pattern of *Salmonella* from poultry farms in Makurdi showed the 83 isolates tested against 10 commonly used antimicrobial revealed a high prevalence of antibiotic-resistant strains of Salmonella, different sensitivity and resistant patterns according to different sample sources and location. The high occurrence of antibiotic-resistant Salmonella in faecal material poses a significant risk for public health. Since resistant strains can spread to humans through direct contact or via contaminated poultry products. The occurrence of resistant Salmonella on egg washings suggest that eggs can be a vector for conveying antibiotic-resistant bacteria to consumers. This points to the need for practice of strict hygiene in handling and processing eggs. The absence in poultry feeds showed that feed management practices do not contribute to the spread of resistance.

The high occurrence of antibiotic-resistant isolates in Wurukum market suggest that consumers and staffs in this market are exposed to an advanced hazard of resistant bacterial strains which has a significant public health concern. The moderate level of resistance isolates exhibited by Modern market is still noteworthy but less than the risk of exposure in the Wurukum market. The low number of resistant isolates in North bank market, suggest relatively lower exposure compared to the others. while vet shops do not contribute to antibiotic resistance in this study as no resistant, intermediate, or sensitive isolates was recorded.

All isolated *Salmonella* spp were resistant to two or more antibiotics, among tested drugs Ceporex, Nalidixic Acid and Ampicillin were highly resistant by the isolated *Salmonella* which indicates these antibiotics are largely ineffective against the isolates studied. Peflacine and Gentamicin also exhibit high resistance rates although they still retain some sensitivity. Moreover, the presence of intermediate and sensitive cases of Salmonella in our study indicated that some strains are still susceptible to certain antibiotics. This finding suggests that there is a possibility of utilizing specific antibiotics for treating Salmonella infections in poultry, while ensuring proper dosage and adherence to veterinary guidelines. The high sensitivity showed by Ofloxacin suggest it might be more effective in treatment and should be considered as a first-line treatment option for infections caused by these isolates.

Okoli et al. (2006) in their study determined the frequency of isolation of Salmonella and their microbial resistance profile across different commercial poultry feeds sold in Imo State, Nigeria, salmonella isolates were tested against 14 anti-microbial drugs. high rates of resistance (51-100%) against nitrofurantoin, ampicillin, tetracycline and ceftriazone, while moderate rates (31-50%) were recorded for chloramphenicol, ofloxacin and cotrimoxazole and low resistance rates (1-30%) against ciprofloxacin and amoxicillin clavulanate (Augumentine), while zero resistance was demonstrated against pefloxacin, gentamicin, streptomycin and nalidixic acid.

Similarly, Parbati et al. (2017) quoted a performed antibiogram study of 72 isolates of Salmonella from poultry in South Korea against 13 antimicrobial drugs available in the market. About 57% of the isolates were resistant of nalidixic acid (NA), 38.9% to amplicilin (AMP), 34.7% to streptomycin (SM), 27.8% each to carbanicillin (CB) and etracycline (TE) and 18.1% to kanamycin (CA). There were less than 10% of the strains susceptible to trimethoprim (ST)

and cefalotin (CF). The most frequent multiple resistant pattern was to AMP, CB, KA, SM, TE, and NA (26.4%).

While Nguyen et al. (2021) isolated salmonella from 181 samples from poultry in poultry farms and households of the Mekong Delta, Vietnam. The isolates were examined for antibiotic resistance against 14 antibiotics and most of the isolates exhibited resistance to 1-9 antibiotics. The isolates were relatively resistant to chloramphenicol (62.98%), tetracycline (55.80%), ampicillin (54.14%), and sulfamethoxazole/trimethoprim (53.04%). Sixty-two multiple resistance patterns were found in the isolates, with ampicillin-cefuroxime-chloramphenicol-tetracycline sulfamethoxazole/ trimethoprim being the most frequent (7.18%).

The common antibiotic classes used in the poultry industry especially fluoroquinolones and penicillin may no longer be effective in treating Salmonella infections because of the high resistance of these salmonella strains. This raises concerns about the potential impact on public health, as the treatment options for Salmonella infections in humans may become limited. This resistance decreases the accessible treatment options for infections caused by these resistant strains. WHO (2017) stated that antimicrobial resistance threat decreases the ability to successful treatment for numerous infectious diseases while simultaneously increases health risks for vulnerable patients for medical procedures.

5.0 CONCLUSION

In conclusion, the survey of selected poultry and poultry feeds in Makurdi for Salmonella and their antibiogram pattern provides valuable insights into the prevalence of Salmonella contamination and the antibiotic resistance patterns in the poultry industry. The findings of this study have several implications for food safety, public health, and the poultry production sector.

The high prevalence of antibiotic-resistant Salmonella strains is a significant concern. It emphasizes the urgent need for responsible antibiotic use practices in the poultry industry to combat the development and spread of antibiotic resistance. This calls for improved antibiotic stewardship, adherence to veterinary guidelines, and continued monitoring of antibiotic resistance patterns in Salmonella strains.

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