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Isolation And Identification Of Salmonella Spp. And Shigella Spp. From Different Poultry Feeds, Droppings And Drinking Water Used In Poultry Farms In Ishiagu, Ebonyi State

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Abstract: ***The study was aimed at isolation and identification of Salmonella spp. and Shigella spp. from poultry feeds, drinking water and droppings used in different farms in Isiagu, Abonyi State. A total of sixty (60) samples were collected from four (4) poultry farms and were analyzed using standard bacteriological methods. The mean bacterial load showed that poultry droppings had the highest load with colonies 8.7 ± 1.15^{a} $x10^{5}$ followed by the feeds 6.9 ± 1.73^{b} $x10^{5}$ and drinking water 6.5 ± 1.15^{b} $x10^{5}$ respectively. The overall prevalence of Salmonella spp. and Shigella spp. in these samples are 60% and 40% respectively. The prevalence of Salmonella spp. are 60%, 70% and 50% whereas the prevalence of Shigella spp. are 40%, 50% and 30% respectively. The two isolates showed varying degrees of resistance to antibiotics tested and completely susceptible to ciprofloxacin and augmentin. The result of this study suggested that these samples are sources of Salmonella spp. and Shigella spp. in poultry farms as well as showing multidrug resistance. Their presence pose a significant public health problem, hence there is need for effective preventive measures in processing and handling of these samples.***

Keywords: Salmonella spp., Shigella spp., poultry feeds, bacterial load, antibiotic resistance

I. INTRODUCTION

Poultry industry is an important business that increases the economy of any nation. The industry is hindered with the outbreak of disease leading to losses in the production of chickens and eggs. Microorganisms that are implicated are food borne pathogens which include *Salmonella* and *Camplobacter spp* which are the most common causes of human food borne bacterial diseases linked to poultry (Hafez, 2005). Other organisms that can be implicated are *Escherichia coli, Enterococcus, Proteus, Clostridium, Providencis, Lactobacillus,* fungus and parasites. However, the pathogens that are discharged from the chicken, contaminate the litter, feed, water and nearby birds (Islam *et al*., 2014). The rapid growth of the poultry industry has resulted in the production of massive quantities of poultry wastes (Islam *et al*., 2014). Poultry litter is used as manure for crop cultivation (Madaki Esther Chat *et al*., 2019) as well as mushroom farming. The application of poultry droppings to soil provides nutrients for the crops growth as well as organic matter for soil conditioning but this can pose danger to public health especially when the crops are eaten raw (Food and Agriculture Organization, 2013).

Shigella is a genus of gram-negative, facultative, anaerobic Enterobacteriaceae that includes *S. dyseenteriae, S. flexneri, S. boydii and S. sonnei* (Shi *et al*., 2014). *Shigella* species have highly evolved in invasive systems that enable the bacteria to invade and multiply within the human intestinal epithelia, ultimately leading to severe inflammatory colitis called bacillary dysentery or shigellosis (Shi *et al*., 2014). Shigellosis in chickens was first reported in 2004 (Shi *et al*., 2004).The main sign of *Shigella* infection is diarrhea, which often is bloody and very contagious. The natural hosts of *Shigella* are conventionally humans and other primates (Shi *et*

al., 2004), however, reports of *Shigella* infection in new hosts including monkeys, rabbits, calves and piglets have emerged (Pan *et al*., 2006; Jiang *et al*., 2005; Priamukhima *et al*., 1984 and Mattrelli *et al*., 1998). *Shigella* infection has become a public health problem since *Shigella* species isolated from human or chicken have identical biological and serological characteristics (Xu *et al*., 2004), hence, care should be taken to avoid cross transmission from man to chicken or vice vasa. Poultry feeds are contaminated during processing by handling, mixing of ingredients and exposing the raw materials and finished products to the atmospheric microorganisms (Madaki Esther Chat *et al*., 2019).

Salmonellosis is one of the food borne diseases in the poultry industry and this is transmitted through meat, meat products, eggs and egg products (Madaki Esther Chat *et al*., 2019). This is as a result of contamination through direct result of animal infection or faecal contamination during processing (Hossain *et al*., 2011).

The evolution and spread of anti-microbial resistant bacterial strains have become a global problem and could possibly be described as threat to public health (Bush, 1997). These resistance could be as a result of misuse of antibiotics, hence, there is need to monitor frequently the susceptibility patterns of bacteria. Antibiotics are used in poultry farms for growth promotion, prophylaxes or therapeutics (Berchieri *et al*.,1989), thus, most of these antibiotics are not fully absorbed in the chicken gut and up to 90% of the administered dose can be excreted in the feces (Kumar *et al*., 2005b). Since litter from farms is used as organic manure, hence, the effect of antibiotics and their potentials on humans and the environment is a public health problem (Boxall *et al*., 2003). Animal feces are potential source of antibiotic-resistant bacteria (Nsofor and Iroegbu, 2013), hence, if released into the environment, resistant strains of organisms may contaminate water and food sources and can be threat to human health (Roy *et al*., 2009). Ishiagu community in Ebonyi State is an agricultural area in which they earn their source of income from poultry farming. Although, there is scarcity of information on the isolation of *Shigella* and *Salmonella* species from poultry feed, droppings and water in Ishiagu, Ebonyi State, hence, there is need to isolate and identify *Salmonella spp*. and *Shigella spp*. from poultry feed, water and droppings used in farms in Ishiagu, Ebonyi State.

II. MATERIALS AND METHODS

COLLECTION OF SAMPLES

A total of 60 samples (20 samples of poultry feed, droppings and drinking water) each were collected from four (4) different commercial broiler/layer poultry farms. The farms were categorized as Amaokwe poultry farm (Farm A), Amaokwe poultry farm (Farm B), Amaeke poultry farm (Farm C), and Ngwogwo poultry farm (Farm D). All samples were collected under aseptic conditions. The different samples were collected in sterile polythene bags and transported immediately to the Department of Applied Microbiology and Brewing, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria for laboratory analyses.

PREPARATION OF MEDIA

All media were prepared according to the manufacture's instruction and autoclaved at 121ºC for 15 minutes.

PREPARATION OF SAMPLES

About 1ml of drinking water and 1g each of droppings and feeds were weighed and mashed into a test tube containing 10ml of sterile peptone water (Al-Abadi *et al*., 2011). This was mixed evenly in order to homogenize the mixture and labeled as the stock. A ten-fold dilution of the homogenates were made with sterile normal saline as diluents (Cheesbrough, 2006). A total of 9ml of the sterile normal saline was measured into ten test tubes. 1ml of the stock was collected using a pipette and serially diluted into the first test tube till the fourth test tube. 10^{-3} and 10^{-4} were used as the dilution factors and 0.1ml was taken from each factor and dispensed into a sterile petri-dish before the prepared media were poured. This was swirled properly and allowed to gel. After gelling, the petri dishes containing *Salmonella-Shigella* agar were incubated at 37^{o}C for 24hrs. After incubation, the representative colonies were identified following standard microbiological methods.

III. CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATES

All the organisms isolated were sub-cultured in nutrient agar plates to obtain the pure cultures. Gram staining and other biochemical tests were carried out based on the method of Cheesbrough (2006).

INDOLE TEST

Sterile test tubes containing 5ml of tryptophan broth were set on a test tube rack, the tubes were inoculated aseptically and the bacteria growth added into it. The tubes were incubated at 37^{0}c for 24 hours. After 24 hours, 0.5ml of kovac's reagent was added to it and allowed to stand for 5minutes, formation of pink or red colour ring in the reagent layer on the medium (within 10 seconds) indicates positive result. Negative result shows no formation of pink or red colour ring.

METHYL RED TEST

The isolates were grown in 5 ml of MR broth (glucose-phosphate peptone water) and incubated for 24 hours at 37^{0}C. Thereafter, 3 drops methyl red were added into each test tube. A reddish colour was observed on the addition of indicator showing positive result while a yellowish colour showed negative result.

CITRATE TEST

Simon citrate agar was prepared and sterilized into a test tube and slanted. It was allowed to solidify before organism was inoculated on the surface of the solidified Simon citrate agar in the test tube. It was covered with cotton wool and incubated at room temperature for 24 hours. For positive result, there will be visible growth and the medium will be blue while the negative result showed no visible growth and no colour change.

CATALASE TEST

Catalase test was done using a test tube, a clean test tube was placed on the rack, 1ml of hydrogen peroxide solution was poured into the test tube, using a sterile glass rod to remove bacteria growth and immerse it into the hydrogen peroxide solution. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

SUGAR FERMENTATIONS

10ml of peptone water was introduced into 5 sterile test tubes respectively. Three (3) drops of methyl red was added into each of the test tubes, then Durham's tubes were inserted in an inverted position into each of the tubes and sealed with foil before sterilization in autoclave at 121^0c for 10 minutes. 1g of respective carbohydrates; glucose, lactose, fructose, sucrose and mannitol, were sterilized using membrane filter and added into each of the sterilized test tubes that contained the peptone water. Thereafter, the cultured organisms were inoculated into each of the tubes respectively. They were then incubated at 37^0c for 24 hours. Positive result indicates yellow colour while gas production were seen in the Durham's tube.

STANDARDIZATION OF INOCULUM

This was done as documented by CLSI (2010). Pure cultures of identified *Salmonella* and *Shigella* isolates from an 18-hour plate culture was selected. Sterile wire loop was used to pick 2 to 3 colonies of each isolates and emulsified in 5ml of peptone water. Adjustment was made with extra inoculum or diluent, if necessary, until 0.5 McFarland standard was obtained. Fifty microliter of the broth was further transferred into 5ml of Mueller-Hinton broth in a tube.

IV. ANTIMICROBIAL SUSCEPTIBILITY TESTING

In-vitro susceptibility of *Salmonella* and *Shigella* isolates to various routine antimicrobial drugs was tested by the standard disc diffusion technique using guidelines established by NCCLS (2004).

The antibiotic discs were evenly dispensed unto the surface of the inoculated agar plates using a disc dispenser and were gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37℃ for 18 hour. The following 10 antibiotic discs were used; ampicillin (30 mcg), septrin (30mcg), nalidixic (30 mcg), ceporex (10mcg), ciproflox (10mcg), augmentin (30mcg), gentamicin (10mcg), reflacine (10mcg), tarivid (10mcg) and streptomycin (30mcg). The plates were inverted and incubated at 37℃ for 18 to 24h. The diameters of the zone of inhibition were measured with a ruler and compared with a zone interpretation chart (Anyanwu, 2010).

STATISTICAL ANALYSIS

Statistical Package for the Social Science (SPSS) was used for the data analysis. Analysis of variance (ANOVA) was used to compute and arrived at statistical decision.

V. RESULTS

BACTERIAL LOAD OF THE SAMPLES

A total of 60 samples (20 droppings, 20 feeds and 20 drinking water) were collected from four different poultry farms, five (5) from each poultry. The mean number of colonies obtained from this work showed that the Poultry dropping have the highest number of colonies (8.7 ± 1.15^a x10^5) followed by the feeds (6.9 ± 1.73^b x10^5) and drinking water (6.5 ± 1.15^b x10^5) respectively. The result is shown in Table 1.

Types of sample	Average Count (CFU/ML)
Droppings	8.7 ± 1.15^a x10^5
Feeds	6.9 ± 1.73^b x10^5
Drinking water	6.5 ± 1.15^b x10^5

Table 1: The mean values of the bacterial count (CFU/ml) of the Droppings, feeds and drinking water samples

Results represent mean ± standard errors, (n=3). Means values with different alphabetic superscripts differ significantly within a column based on LSD analysis at $p< 0.05$

PREVALENCE OF SALMONELLA SPP AND SHIGELLA SPP ON THE SAMPLES

The overall prevalence of *Salmonella* spp. in this study was 60%. The prevalence of *Salmonella spp*. in feeds, droppings and drinking water were 60%, 70% and 50% respectively. While the overall prevalence of *Shigella spp*. in these samples were 40%. The prevalence of *Shigella spp*. in feed, droppings and drinking water were 40%, 50% and 30% respectively. The result is presented in tables 2.

No. of Poultry Farm	Samples	Number of Sample	Positive for *Salmonella spp*	Positive for *Shigella spp*
5	Feeds	20	12(60%)±1.15	8(40%)±0.58
	Droppings	20	14(70%)±0.58	10(50%)±1.15
	Drinking water	20	10(50%)±0.58	6(30%)±0.58
Total	3	60	36(60%)	24(40%)

Table 2: Prevalence of Salmonella spp and Shigella spp in Selected Poultry Farms

Results represent mean ± standard errors, (n=3). Means values with different alphabetic superscripts differ

significantly within a column based on LSD analysis at p< 0.05.

MORPHOLOGICAL IDENTIFICATION OF SALMONELLA SPP. AND SHIGELLA SPP

The bacterial isolates were characterized and identified mainly on the basis of their colony morphology and cellular morphology. The result is shown in Tables 3.

Media used	Choromogenesis	Elevation	Shape	Suspected organism
Salmonella -Shigella agar	Colorless	Convex	Round	*Shigella* species
Salmonella -Shigella agar	Black	Convex	Round	*Salmonella* species

Table 3: Morphology, cultural characteristics and staining characteristics of the isolates

BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

The isolates were further identified and characterized using Gram stain, sugar fermentation test and other biochemical tests. All the *Salmonella spp*. isolates fermented Fructose, glucose and mannitol and produced acid and gas or only acid. No fermentation was seen in lactose and sucrose while all the *Shigella spp*. isolates fermented sucrose, glucose and mannitol and produced acid and gas or only acid. No fermentation was seen in lactose and fructose. The result is presented in table 4.

Isolates	Gram reaction	Mannitol Fermentation	Glucose fermentation	Sucrose Fermentation	Lactose Fermentation	Fructose Fermentation	Catalase Fermentation	Indole test	Methyl red	Citrate test
Salmonella Spp.	-ve rod	Acid and gas	Acid and gas	-	-	Acid and gas	+	-	+	+
Shigella Spp.	-ve rod	Acid only	Acid only	Acid and gas	-	-	+	+	+	-

Table 4: Biochemical Characteristics of Salmonella spp and Shigella spp

ANTIBIOTIC RESISTANCE PATTERN OF THE ISOLATES

The isolates were subjected to different antibiotics. The results are presented in the table 5.

Antibiotics	Percentage of isolates resistance to antibiotic *Salmonella* (No.=10)	*Shigella* (No.=7)
SXT (30mcg)	3(30%)±0.58	3(42.9%)±0.00
NA (30mcg)	2(20%)±0.58	7(100%)±0.58
CEP (10mcg)	9(90%)±0.58	5(71.4%)±1.15
CPX (10mcg)	1(10%)±0.00	0(0.0%)±0.00
PN (30mcg)	9(90%)±1.15	7(100%)±1.15
AU (30mcg)	0(0%)±0.58	2(28.6%)±0.58
CN (10mcg)	4(40%)±0.00	3(42.9%)±0.58
PEF (10mcg)	3(30%)±0.58	1(14.3%)±0.00
OFX (10mcg)	0(0%)±0.00	0(0%)±0.00
S (30mcg)	3(30%)±0.58	1(14.3%)±0.00

Results represent mean ± standard errors, (n=3)
Keys: SXT=Septrin, Na=Nalidixic Acid, CEP=Ceporex CPX=Ciproflox, PN=Ampicillin, AU=Augmentin, CN=Gentamycin, PEF=Reflacine OFX=Tarivid, S=Streptomycin.

Table 5: Antibiotic resistance pattern of Salmonella spp and Shigella spp

VI. DISCUSSION

Poultry industry has become the source of meat and animal wastes which are used as manure for plant production but this has been jeopardized as a result of pathogenic organisms which are of public health concern. In this study, the presence of *Salmonella spp* and *Shigella spp*. from poultry droppings, feed and drinking water were investigated. The bacterial load (cfu/g) of droppings, feed and drinking water were $8.7x10^3$, $6.9x10^5$ and $6.5x10^5$ respectively (table1). This study is in agreement with the work done by Roy *et al*. (2017) where they observed bacterial load of *Shigella spp*. from poultry feed to range from $3.2x10^4$ cfu/g to $1.1x10^6$ cfu/g while *Salmonella spp*. was $7.0x10^3$ cfu/g also Deke Adegunloye (2006) observed these organisms in poultry faeces to range from $4.5x10^7$ to $7.0x10^7$ cfu/g. This high bacterial load observed could be as a result of contamination of feed during processing or cross contamination from the feed to drinking water. This could also be as a result of improper disposal of droppings as well as hygienic practices in the poultry house. Therefore, storage condition, packaging and handling should be maintained properly (Roy *et al*., 2017) as well as cross contamination should be minimized. The present study revealed the overall prevalence of *Salmonella spp*. and *Shigella spp*. as 60% and 40% respectively. The individual prevalence of *Salmonella spp*. and *Shigella spp*. in faeces, droppings and drinking water are 60%, 70% and 50% respectively while 40%, 50% and 30% respectively (table 2). The prevalence of *Salmonella* spp. in the present study was in close agreement with the works of Islam *et al*., 2014 and Mishra *et al*., 2002. The study is also in agreement with the work of Yhiler and Bassey, (2015) who reported an isolation rate of 59.1% of *Salmonella spp*. from cloacal swab of poultry in Calabar Cross River State, Nigeria. Also Umeh and Enwuru (2014) reported 52.5% isolation rate of *Salmonella spp* from chicken faecal samples in Owerri metropolis Imo State, Nigeria. However, the prevalence rate of *Salmonella spp*. obtained in this study was higher compared with the results of Rodriguez *et al*. (2015) who reported a prevalence of 17.4% of *Salmonella spp* in broilers at Ibague, Colombia; Musa *et al*.(2014) who obtained 9% prevalence from water samples in a study on isolation and antibiogram of *Salmonella spp*. from water and feed in selected poultry farms in Zaria; Madaki Esther Chat *et al*.(2019) who reported 33.33% of *Salmonella spp*. from poultry feeds and droppings in farms visited in Kaduna State; Al-Khayat and Khammas (2016) who reported a prevalence of 10.4% of *Salmonella spp*. from layers and

broilers in Baghdad and Ejeh *et al*. (2017) who reported a prevalence of 10% and 12% of *Salmonella spp* from local chicken and broilers respectively. Also Garcia *et al.* (2011) reported a lower prevalence rate of Salmonella from laying hens in Aquidauana, Brazil as well as other studies carried out in other parts of the world (Akond *et al.* (2012). The prevalence of these organisms could be as a result of environmental condition, hygienic practices and cross infection with other organisms, as they are capable of causing acute and chronic infections in birds. The presence of *Salmonella* species in the feed is of public health concern, this is because its transmission in the environment has been shown to be cyclic (Madaki Esther Chat *et al*. (2019) and poultry feeds had been viewed as important links for contamination in poultry (Maciorowski *et al*., 2006). The presence of *Shigella spp*. in these samples are of public health problem as they are pathogenic to chickens as well as serious enteric pathogens (Periasamy *et al*., 2013), hence leading to deaths of chicken and economic loss to farmers.

In the present study, it was observed that the isolates of *Salmonella* and *Shigella* species showed varying degrees of resistance to some antibiotics tested (table5). Resistance of 90% of *Salmonella spp* to both ceporex and ampicillin was observed while *Shigella* species showed 71.4% and 100% respectively to these antibiotics. *Shigella* species showed 100% resistance to nalidixic acid. *Salmonella* and *Shigella* species showed completely susceptible to ciprofloxacin and augmentin. The susceptibility of *Salmonella* and *Shigella* species to ciprofloxacin and augmentin is well understood because these drugs are widely used in poultry and human health for the treatment of typhoid fever and other enteric diseases. The susceptibility of *Salmonella* and *Shigella* species to ciprofloxacin was in line with work done by Roy *et al*. (2017) who observed 70% and 55% to ciprofloxacin respectively. Generally, it was observed in this study that multiple antibiotic resistance was common among *Salmonella* and *Shigella* species. This multiple antibiotic resistance may be due to excessive and uncontrolled use of antibiotics in poultry (Velge *et al*., 2005; Nsofor *et al*., 2013; Adeyanju and Ishola, 2014). Salihu *et al*. (2014) further explained that the excessive use of antibiotics in poultry results from being freely available and readily affordable. Antibiotic resistance is a worldwide problem in both human and veterinary medicine (Ejeh *et al*., 2017) hence, varieties of factors have been identified as the cause of bacterial resistance. Van den Bogaard *et al*. (2001) pointed out that usage of antibiotics was the most significant factor responsible for antimicrobial resistance in bacteria as well as overcrowding and poor sanitation. Hence, this could be the reason for emerging antibiotic resistance in Nigeria (Ejeh *et al*., 2017), since antibiotics are used excessively in poultry and livestock in Nigeria without regulation (Raji *et al*., 2007; Olonitola *et al*., 2015; Geidam *et al*., 2012).

VII. CONCLUSION

In this present study, it was observed that these samples examined are potential reservoir for pathogenic organisms which are shown to be resistant to some antibiotics, hence, suggesting a public health problem. However, indiscriminate use of antimicrobials has led to multidrug resistant strains of these organisms, hence, there is need to monitor and advocate the use of ciprofloxacin and augmentin in which they are susceptible, to avoid the emergence of drug resistance. Also, there should be public health awareness to farmers on the proper disposal of poultry wastes to avoid cross contamination within the poultry house and provision of good quality poultry feeds.

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