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Occurrence and Antimicrobial Susceptibility Profiles of *Salmonella* species among Chickens of some Commercial Poultry Farms in Benue State, Nigeria

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Abstract

Salmonella is a facultative anaerobic Gram negative rod, an intracellular bacterium of zoonotic importance. This study aimed at investigating the presence of *Salmonella* in chickens in three local government areas of Benue State. A total of 588 cloacal swabs were made randomly from 264 layers and 324 broilers at Makurdi, Otukpo and Gboko local government areas of Benue State, from March to July 2015. *Salmonellae* were isolated and identified using standard bacteriological techniques namely culture, Gram's staining and biochemical methods. Cultural characteristics of isolates on the used media were observed, pink colonies with or without black centers on xylose lysine deoxycholate agar (XLD) and brilliant green agar (BGA) were considered presumptive *Salmonella* isolates and were later characterized biochemically using standard conventional methods. Thereafter, an *in-vitro* antimicrobial susceptibility testing was conducted on *Salmonella* isolates using the disc diffusion method with six selected, commonly used antimicrobials ampicillin (10 µg), gentamycin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), norfloxacin (10 µg) and amoxicillin (10 µg). Thirteen (13) isolates were identified and confirmed as *Salmonella* species, giving an overall occurrence rate of 2.21% (13/588). Isolates were found to be most susceptible to Norfloxacin and Chloramphenicol. In conclusion, this study revealed the occurrence of *Salmonella* in chickens within the study area, this is of great concern due to possible effect of disease dissemination in chickens, as well as transmission to humans through the food chain,

Key words: antimicrobial profiles, chicken, Nigeria, *Salmonella* species.

INTRODUCTION

Benue State is an Agriculture based State in Nigeria, crop cultivation and livestock rearing are the major sources of livelihood of Benue people. Agriculture contributes immensely to the state economy. *Salmonella* infections are important causes of clinical salmonellosis in poultry and a potential source of food borne illness in humans (Shivaprasad, 2000). Under the family *Enterobacteriaceae*, the genus *Salmonella* is divided into two species (*Salmonella enterica* and *Salmonella bongori*) each with multiple subspecies and serotypes (Lutful, 2010).

Salmonella is a pathogen of zoonotic importance, it causes heavy economic losses in commercial poultry in places like Benue State where control measures are not effective and climatic conditions such as high temperature and humidity favor bacteria multiplication and environmental contamination (Barrow and Freitas, 2011). The epidemiology of salmonellosis is complex, which often makes control of the disease difficult. The epidemiological patterns of prevalence of infection and incidence of disease differ considerably between geographical areas depending on the climatic conditions, management practices and population density (Otto *et al.*, 2000).

Salmonella infection in chickens can occur vertically when chicks are hatched from infected ovary, or from infected eggs that were contaminated during passage through the cloacal faeces (Lutful, 2010). Horizontal transmission of the organisms occurs when chickens ingest feed or water that has been contaminated with faeces of clinically infected birds or other carrier animals (Abdu, 2014). In poultry, most *Salmonella* infections are associated with environmental contamination, most chickens once infected become chronic carriers and continue to shed the organism in their feaces (Raufu *et al*,. 2009).

Generally, poultry farms where biosecurity measures are poorly implemented, horizontal transmission from an infected chicken to a susceptible chicken or flock is more likely to occur (Echioda-Ogbole *et al,*. 2017).

Clinical salmonellosis in chickens varies based on the invading serotypes. *Salmonella* Pullorum and S. Gallinarum are the two common host adapted serotypes that infect chickens. S. Pullorum, the etiological agent of Pullorum disease is most common in infecting chicks and growing birds and is characterized by bacillary white diarrhea with high mortality in broilers and layers. Whereas S. Gallinarum causes fowl typhoid in adult birds (Lutful, 2010). Poultry constitute one of the largest and most important reservoirs of non- host adapted *Salmonella* serotypes such as S. Enteritidis, S. Kentucky, S. Typhimurium, S. Ziga, S. Nima and S. Livingstone which are commonly referred to as paratyphoid *Salmonellae* (Lutful, 2010).

Paratyphoid infections in chickens can be transmitted to humans through the consumption of contaminated poultry and poultry products. In the last two decades, there has been an increase in multi-antimicrobial resistant *Salmonella* strains in humans, this has been associated with indiscriminate use of antimicrobial agents in poultry and other food animal production, and it is a major public health issue (Lynch *et al,*. 2006).

"The gold standard" for diagnosing salmonellosis requires the isolation and identification of the organism using conventional bacteriological technique or molecular detection using PCR. Early detection of *Salmonella* infections is very paramount in the prevention of clinical salmonellosis as well as disease dissemination. In view of the above, this study was undertaken to estimate the occurrence rate of *Salmonella* in chickens and to determine the susceptibility of isolates to commonly used antimicrobials in the study area.

MATERIALS AND METHODS

Sample collection and handling

A total of 588 cloacal swabs were randomly made, comprising of 264 layers and 324 broilers from commercial poultry farms and household chickens in Makurdi, Otukpo and Gboko local government areas of Benue State, Nigeria from March to July 2015. The cloacal swabs were collected from live chickens using sterile cotton tipped swabs as recommended by International Office of Epizootics (OIE, 2012). Samples were immediately placed in a flask with ice packs and transported to the Veterinary Medicine laboratory, University of Agriculture Makurdi and stored at 4^{o}C until required for isolation and identification of *Salmonella*.

Isolation and identification of *Salmonella*

Salmonellae were isolated according to standard methods described by ISO 6579 (2002) and OIE (2011). Each cloacal swab collected was analyzed independently. In brief, the cloacal swabs collected in nutrient broth were incubated at 37^{o}C overnight for 24hr. One millilitre of the non-selective pre-enriched culture was inoculated into 9 ml of selenite-F broth for selective enrichment and incubated at 37^{0}C for 24 ± 3h. Selective plating was carried out by streaking a loopful of pre- enriched broth onto plates of brilliant green agar (BGA) and xylose lysine deoxycholate (XLD) agar and incubated at 37^{0}C for 24 ± 3h. All plates were examined for presence of typical colonies of *Salmonella,* which are red colonies with black centres on xylose lysine deoxycholate (XLD) agar and pink colonies surrounded by a red medium on brilliant green agar (Antunes *et al.,* 2003; OIE, 2011).

Six (6) presumptive *Salmonella* colonies were sub cultured to purify on nutrient agar and then incubated at 37^{o}C for 24 ± 3h followed by phenotypic characterization using Gram's staining and conventional biochemical tests such as triple sugar iron test, methyl red test, indole test, urease test, citrate test, sugar fermentation test and motility test as prescribed by Cowan and Steel (1993); ISO, (2002); OIE, (2012).

In-vitro antimicrobial susceptibility testing

In-vitro antimicrobials susceptibility testing was conducted on all the positive *Salmonella* isolates using the disc diffusion method as described by Bauer *et al.* (1966) and Clinical Laboratory Standards Institute (2014). Six selected therapeutic antimicrobial were used for the study, includlng ampicillin (10 µg), gentamycin (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), norfloxacin (10 µg) and amoxicillin (10 µg). Three discrete colonies of each *Salmonella* isolates were inoculated on the surface of Mueller Hinton Agar plate (Titan Biotech, India) and sterile forcep was used to place the paper impregnated antimicrobial discs (Oxoid Ltd, UK) centrally and incubated at 37^{o}C for 24hr (Clinical and Laboratory Standards Institute, 2014).

The zones of growth inhibition around each of the antimicrobial discs were measured with a millimeter ruler to the nearest millimeter and the results were interpreted as follows: clear zones of no growth around the edges of the antimicrobial discs of less than13 mm was described as resistant,

absence of growth around the edges of the antimicrobial discs to between 13-17mm was described as intermediate susceptible and absence of growth around the discs to about 18mm or more was described as susceptible (Clinical and Laboratory Standards Institute, 2014).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used for data analysis. Simple descriptive statistics such as percentages and tables were used to express the rate of occurrence of *Salmonella*. Incidence was evaluated as number of samples positive to *Salmonella* isolation from the total sample analyzed. Chi-square test was used to check level of significant difference of *Salmonella* occurrence rate in the three locations of the study area and in layers and broilers. P<0.05 was considered significant.

RESULTS

Overall, thirteen (13) *Salmonella* isolates were obtained from the five hundred and eighty eight (588) cloacal swabs, giving a prevalence of 2.21%. Of this, four (4) isolates were obtained from two hundred and eighty eight cloacal swabs analyzed from Makurdi, nine (9) *Salmonella* isolates were gotten from the one hundred and fifty (150) samples analyzed from Otukpo, the one hundred and fifty samples from Gboko yielded no *Salmonella* growth (Table1).

Table 1: Percentage distribution of *Salmonella* isolates from cloacal swabs of chickens in three Locations in Benue State.

Location	No of samples	No of isolates	prevalence %
Makurdi	288	4	1.39
Otukpo	150	9	6
Gboko	150	0	0
Total	588	13	2.21

Also, there was statistical significant difference (p< 0.05) in number of isolates obtained from layers as compared to that gotten from broilers as shown in Table II.

Table II: Percentage distribution of *Salmonella* species isolated from layers and broilers in three locations in Benue State.

Type of Chicken	No of samples	No positive	prevalence (%)
Broilers	324	2	0.6
Layers	264	11	4.1
Total	588	13	2.21

Table III shows result of the *in-vitro* antimicrobials susceptibility testing of *Salmonella* isolates to six selected antimicrobials. Isolates were more sensitive to Norfloxacin (92%) and completely resistant to Ampicillin (100%).

Table III. *In - vitro* antimicrobial susceptibility testing of *Salmonella* isolates to six selected antimicrobials.

Antimicrobial	Susceptibility (%)	Intermediate (%)	Resistant(%)
Gentamicin 30 µg	61.5	0	38
Tetracyclin 30 µg	31	23	46
Chloramphenicol 30 µg	69.	7.7	23
Ampicillin 10 µg	0	0	100
Norfloxacin 10 µg	92	0	7.7
Amoxicillin 10 µg	7.7	15	76

Key: % = percentage, µg= microgram

DISCUSSION

This study reports the first isolation of *Salmonella* from cloacal swabs of live chickens in the study area. A relatively low prevalence of *Salmonella* was recorded among commercial chickens in Benue State, with Otukpo having the highest prevalence as presented in Table 1. The high isolation rate recorded in Otukpo ($p<0.05$) could be attributed to the poor sanitary condition of the town visa-a-vis the poor biosecurity measures of poultry and household farms in the area. The 2.21% occurrence rate of *Salmonella* in this study was not cheering. The finding of this study concurs with the report of OIE (2012) which reported that isolation of *Salmonella* from feces and cloacal swabs of chickens although possible but not rewarding. This is because chickens can become chronic carriers of *Salmonella* and thus excrete the organism intermittently (Raufu *et al.*, 2009; Ameh *et al.*, 2016). And that the preferred samples for *Salmonella* isolation are Post- mortem tissues such as liver, spleen, kidney, gallbladder, heart, lungs, alimentary tract or ileo-caecal junction, testes and ovaries (OIE, 2012). The 2.21% incidence of *Salmonella* recorded in this study is relatively similar to 1.6% prevalence of cloacal *Salmonella* reported in Brazil (Moraes *et al.*, 2016), 4% in Spain (Garcia *et al.*, 2011) and 4.4% in India (Singh *et al.*, 2013). But far lower than the 19% reported in Iraq (Al-Abadi and Al-Mayah 2012). The high prevalence of 19% reported in Iraq could be attributed to variation in *Salmonella* status among countries and locations, culture media, as well as isolation methodology used. A study conducted by Moraes *et al.* (2016) used two different isolation methods, the conventional culture method and quantitative PCR to isolate *Salmonella* from cloacal swabs of chickens and recorded a prevalence of 1.6% and 20.8% respectively in Brazil. The difference in isolation rate in their study is a clear pointer that method used in the isolation of an organism affects the outcome of the result. This could be attributed to the sensitivity of the qPCR in detecting the organism. In this study, there was statistical significant difference ($p< 0.05$) in the number of isolate based on the type of chicken, with layers (6%) having the highest number of isolates as compare to broilers as seen in Table II. This finding is in agreement with report of Bura *et al.* (2014) who reported 15% prevalence of *Salmonella* in layers in Tanzania. This may be attributed to horizontal transmission of the organism between flocks or from a clinical infected bird to a susceptible chicken as it relates to longer stay of layers.

All the *Salmonella* isolates in this study did not ferment lactose and sucrose but did ferment glucose and mannitol with variable production of acid with or without gas production. With the exception of one isolate, all other isolates fermented maltose. Furthermore, isolates were indole negative, methyl red positive, triple sugar iron positive, citrate positive and urease negative. These findings are in accordance with report of OIE, (2012).

The antimicrobial susceptibility testing results in this study showed varying degree of resistance and susceptibility of isolates to the antimicrobial agents tested. All the isolates in this study showed complete resistance to ampicillin (100%), 76% resistance to amoxicillin was also recorded. This result is in line with the report of Reda *et al.* (2011), reported that except for gentamicin and norfloxacin, the *Shigella* and *Salmonella* isolates in their study showed high level of resistance to ampicillin, amoxicillin, tetracycline and chloramphenicol. Also, Agbaje *et al.* (2010) reported identical patterns of resistance of *Salmonella* isolates to the antimicrobial agents used in their study, among which is ampicillin. Resistance to antimicrobials such as ampicillin and amoxicillin could be associated with prolonged and indiscriminate use by poultry owners and clinicians. Although *Salmonella* isolates in this study were sensitive to Norfloxacin (92.3%) and Chloramphenicol (72.4%), the use of Chloramphenicol in food animals such as chickens is not recommended. And generally, treatment of clinical salmonellosis in chickens with antimicrobials is not advisable because a variable percentage of chickens once infected, remain carriers and shed the organism intermittently in feces, with an impending risk of environmental contamination (Raufu *et al.*, 2009).

CONCLUSION

This study reports the occurrence of *Salmonella* from live chickens in the study area at 2.21%. The existence of *Salmonella* in commercial chickens in the study area is of great concern due to possible outcome of environmental contamination of water and feed which can enhance horizontal transmission of the organism. In order to prevent vertical and horizontal transmission of *Salmonella* in chickens, we therefore recommend implementation of proper biosecurity and biorisk management measures in poultry farms as well as regular vaccination of chickens against *Salmonella* infections.

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Conflict of interest
The authors declare that they have no conflict of interest.

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