IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 5 Ver. I (May. 2016), PP 18-22
www.iosrjournals.org

Isolation And Antimicrobial Susceptibility Studies Of Salmonellaspecies, From Chickens In Gwagwalada And Kwali Area Councils, Abuja, Federal Capital Territory, Nigeria

James A. Ameh, Samuel Mailafia, Olatunde H. Olabode, Bridget J. Adah, Martha Echioda, Godspower R. Okoh And Agnes Ejeh
Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, F.C.T- Nigeria

Abstract: *Isolation and antimicrobial susceptibility testing of Salmonella Species from live and dead chickens in Gwagwalada and Kwali area councils of Abuja, was studied to establish the prevalence and possible treatment regimen forSalmonella in the study area. Five hundred (500)samples of bothfaecal (180) and visceral organs (320) were collected from chickens in poultry farms and slaughter houses between Mayand August 2015.Salmonellae were isolated, identified and characterized using standard methods. Isolates were further subjected toantimicrobial susceptibility testing using disc diffusion method.The occurrence of Salmonellaspeciesisolates revealed 8%(40) andthese isolates were most susceptible to Ciprofloxacin and Gentamicin.Serotyping of isolates for effective control of outbreaksusing vaccines is thus suggested, while farmers and poultry attendants should ensure strict hygienic practice.*

Keywords: *Salmonella;antimicrobial susceptibility testing;poultry farms and Slaughter houses.*

I. Introduction

Avian *Salmonella* infections are important causes of clinical disease in poultry and a potentialsource of food borne transmission of *Salmonella* in humans (Shivaprasad, 2000).*Salmonella*organism are classified under the family Enterobacteriaceae, Genus *Salmonella* which is a gram-negative, non-spore forming, aerobic or facultative anaerobic rods that aremostly motile with exception of *S. gallinarum* and *S. pullorum* which are non-motile, (Harris *et al.,* 1997; Rao, 2000; Nwachukwu and Nwiyi, 2011). There are over 2,400 serologically different variants/serotypes of *Salmonellae* which inhabits the gastrointestinal tract of humans and animals (Faruk *et al*., 2005).Theseorganisms aretransmitted mainly through ingestion of feed or water contaminated by feaces of clinically infected birds or other animals and human carriers (Shivaprasad, 2000; Abdu, 2007).Avian *Salmonella* infections have been eradicated from commercial poultry in many developed countries of Western Europe, USA, Canada, Australia and Japan where intensive poultry industry operates (OIE, 2005). In Africa, fowl typhoid and pullorum disease caused by *Salmonellagallinarum* and *Salmonellapullorum* respectively, have been reported in many countries including Nigeria(Okoli *et al.,*2006; Ajayi and Egbebi, 2011).The disease has been reported in chickens with clinical signs of septicaemia, diarrhoea, enteritis and is characterized by drop in egg production and increase Mortality (Jensen *et al*., 2003).A tentative diagnosis of Salmonellosis is based on flock history, clinical signs, mortality and lesions. However,a definite diagnosis requires the isolation and identification of *Salmonella*. In addition, various serological tests such as serum plate agglutination test, rapid agglutination test, tube and micro titre agglutination test and Polymerase Chain Reaction canbe used to detect *Salmonella* infections (Shivaprasad, 2000).However, reports of increasing outbreaks associated with avian salmonellosis have recorded despite the use of vaccines (Barrow and Freitas, 2011).Therefore, thisstudy employed the use of conventional methods for identification and characterization of *Salmonella*isolatesin order to establish the occurrence and burden of the disease as well as antimicrobial susceptibility testing of Avian*Salmonella* in poultry farms and slaughter houses in the study area.

II. Materials And Methods

Study area

Gwagwalada and Kwali are amongst the six area Councils in the Federal Capital Territory Abuja, Nigeria. Gwagwalada is located between latitude $8^0 45^1$N of the equator and longitude $6^0 45^1$ and east of the Greenwich meridian, with land mass of 1043Km^2 and ten wardshaving an annual rainfall of approximately 368mm, temperature of 25^0C – 35^0C yearly and a population of 157, 770 at 2006 census.Abuja is located in the North Central region of Nigeria and shares boundaries with KogiState to the South East and South West, NigerState to the North Westand NasarawaStates to the North(Anon, 2007).

Sample collection

Five hundred samples comprising of 180 feacal samples from 45 commercial layer flocks (n= 4 for each farm) and 320 visceral samples (intestine, liver and spleen) (n = 20)were collected from two poultry

slaughter outlets in Gwagwalada and Kwali area council. This sample collection was conducted between May and August 2015 based on convenience random sampling method. Ten gram each of liver, spleen and intestine were aseptically collected from slaughtered chickens and placed in universal bottles containing 5mls of nutrient broth (Laboratarios Britania, Buenos Aires, Argentina), while deep cloacal and ceacal tonsil swabs were also collected and placed in 5mls of nutrient broth.All samples were immediately transported on ice to the Veterinary Microbiology Laboratory of Faculty of Veterinary Medicine, University of Abuja and for processing and storage until use.

Isolation and Identification of *Salmonella species*

Isolation of *Salmonella* was conductedin accordance with standard methods as described by Mdegela *et al*.(2000) and Murugkar *et al.* (2005). Liver, spleen and intestine (10g) samples of the same chicken were homogenized by stomacher; both the homogenate and swabs were aseptically innoculated into 10ml of selenite-F broth for selective enrichment and incubated at 37^0c for 24hrs. A loopful from each of the enriched broths were streaked onto plates of MacConkey(Oxoid Ltd, UK, without salt) and blood agar were incubated at 37^0C for 24hrs. Selective plating was performed using *Salmonella*-Shigella Agar (SSA) and Deoxycholate Agar (DCA). All the plates were examined for presence of typical colonies with black centres on *Salmonella* Shigella Agar (SSA) and red colonies with black centres on Deoxycholate agar (DCA). Suspected colonies were confirmed positive using conventional biochemical methods (indole (I), methyl red (MR), vogusproskeur (Vi),citrate(C) triple sugar iron (TSI) and urease test) and the results obtained were recorded and interpreted as stated by Proux *et al*. (2002); Parmar and Davies, (2007).

Antibiotic sensitivity testing

An *in-vitro*antibiotics sensitivity test wasconducted on positive *Salmonella* isolates using disc diffusion method as described by James (2009); Bauer *et al.*(1966) with a panel of eight (8)therapeutic antibiotics impregnated disc namely: Chloramphenicol, CH (30 µg), Gentamicin, GN (10 µg), Norfloxacin, NO (10 µg), Ciprofloxacin, CP (10 µg), Tetracycline TET (30 µg), Amoxicillin clavulanate, AU (30 µg), Ampicillin, AM (30 µg), Nalidixic acid, NA and Nitrofurantoin, NF (30 µg). Briefly, a MacFarland 0.5 standardized suspension of the bacteria in 0.8% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar (Oxoid) with a sterile swab loop. A ring of disks (Mast Diagnostics, UK) each containing single concentrations of antimicrobial agent was placed onto the inoculated lawn and incubated at 37°C for 24h. Clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler. The diameter of the zones was read using aninterpreting chart for zone sizes in accordance with standard methods described by National Committee for Laboratory Standards (2004).

Statistical analysis

The numbers of positive *Salmonella* isolates were expressed using simple descriptive statistics such as percentages and frequencies.

III. Results

Out of the 500 samples analysed 8% (40)were positive for *Salmonella*.Out of the forty (40) isolates, six (6) isolates were from 180 feacal samples, representing a percentage distribution of 1.2%, while 34 isolates were from 320 visceral samples with a percentage distribution of 6.8% as shown in Table I below. The biochemical characteristics of isolates to various chemicals and sugars are as shown in Table II.

The result of antimicrobial susceptibility testingshowed isolates were only sensitive to Ciprofloxacinand Gentamicin, but were resistantto Neomycin, Ampicillin, Chloramphenicol, Tetracycline, Amoxicillin and Orfloxacin.

Table 1: **Distribution of *Salmonella*species obtained from chickenfeacal and visceral samples in Kwali and Gwagwalada Area Councils, Abuja-FCT**

Type of samples Collected	No. of positive Samples (%)	No. of negative Samples (%)	Total
Faeces	6 (1.2)	174 (34.8)	180 (36)
Visceral	34 (6.8)	286 (57.2)	320 (64)
Total	40 (8)	460 (92)	500 (100)

Table II:Cultural and Biochemical Characteristics of *Salmonella*isolates obtained from visceral organs in the study area

Sample	No of tested sample	No of positive	No of negative	Butt	TSI Slant	H_2S-prd.	TSI GLU	TSI Lact	Indole	catalase	Cit.	Oxidese	MR	VP	Sucrose	Mannito l	Detec-tion(%)
LMC_1	20	4	16	(yellow)	(red)	+	A	-	-	+	+	-	+	-	-	A	0.04
LML_2	20	5	15	(yellow)	(red)	+	A	-	-	+	+	-	+	-	-	A	0.05
$LMCT_3$	20	10	10	(yellow)	(red)	+	A	-	-	+	+	-	+	-	-	A	0.1
LMS_4	20	15	5	(yellow)	(red)	+	A	-	-	+	+	-	+	-	-	A	0.15

KEY: PF_A– PF_D = Faecal Samples MR. = Methyl Red VP = Vogo'sProskuer, Cit =Citrate, Glu = Glucose, Lact = Lactose
LM = Local Market, C_1= Caecal, L_2= Liver, CT_3= Caecal and S_4= Spleen, A=Fermented + Positive, =Negative, Yellow butt = Acidic (colour changes to yellow due to acid formation), red= Alkaline (colour changes to red due to Alkalinization) H_2S = + (blackning due to H_2S

Table III: Cultural and Biochemical Characteristics of *Salmonella* from chicken feacal samples in Gwagwalada and Kwali Area Council.

Sample	No of tested sample	No of positive	No of negative	Butt	TSI Slant	H^2S-prd.	TSI GLU	TSI Lact.	Indole	catalase	Cit.	Oxidese	MR	VP	Sucrose	Mannit ol	Detec-tion(%)
PF_A	45	2	43	-	-	-	-	+	+	-	-	+	+	A	+	-	0.02
PF_B	45	1	44	-	-	-	-	+	+	-	-	+	+	A	+	-	0.01
PF_C	45	2	43	-	-	-	-	+	+	-	-	+	+	A	+	-	0.02
PF_D	45	1	44	-	-	-	-	+	+	-	-	+	+	A	+	-	0.01

KEY: PF_A– PF_D = Faecal Samples,MR. = Methyl Red, VP = Vogo'sProskuerCit. = Citrate, Mor. = Mortility, Glu.= Glucose,*Sal. Spp.* =*Salmonella*species

Table IV: *In – vitro* antibiotics sensitivity testing of *Salmonella spp*. Isolates from chicken faecal and visceral organs. No of isolates 40

Drug	% sensitivity to*SalmonellaSpp*
Ciprofloxacin	95.0
Gentamicin	87.5
Chloramphenicol	5.0
Norfloxacin	7.5
Tetracyclin	7.5
Amoxicillin	0.0
Ampicillin	5.0
Nalidixic acid	0.0

IV. Discussion

The overall 8% prevalence of *Salmonella* in this study is relatively similar to 11% reported by Fashae *et al*.,(2010). However, Mike *et al.* (2004) and Raufu *et al*. (2010) reported 12.5% and 15% respectively, which was a slightly higher than that obtained in this present study. The variation in the isolation rate might be due to variation in climatic conditions, difference in the management system or better still due to difference in the number of samples in each study, since large sample size caninfluence the chances of obtaining more isolates on culture as previously stated (Mollenhorst *et al*., 2005; Raufu *et al*., 2010). In addition, the difference in study location may play a role in the proliferation and occurrence of the organism, as *Salmonella species* are known to thrive differently in various environmental conditions and seasons (Okoli *et al*., 2006).

In this study, the percentage occurrence of *Salmonella* isolates obtained from chicken fecal samples is lower than that obtained from chicken visceral samples, this findings is in agreement with the report of OIE, (2010) and this is because chickens can become chronic carriers of*Salmonella* organism and thus excretes the organism in their faces intermittently (Raufu, *et al.*, 2010). It is important to note that the visceral samples in this study were obtained from chicken slaughter outlets in the market, therefore the high isolation rates in the visceral organs could be due to environmental contamination. Also, sampling was in various markets with chickens brought in from different locations within the study area that were raised under different management systems such as free range or backyard system with little or no biosecurity practice. Hence, a potential probability of increase occurrence of the organism, as unhygienic and poor sanitary practices predisposes to infection(Mike *et al*., 2004; OIE, 2010). All the isolates in this study post biochemical characterization showed typical reactions of *Salmonella* which is indole negative, methyl red positive, voges-Proskauer negative, citrate positive, triple ion sugar positive and oxidase negative as previously described by OIE, (2010).

Antimicrobial susceptibility testing in this study showed sensitivity of isolates to Ciprofloxacin and Gentamicin with an associated high rate of multiple antibiotic resistances. Thisis in agreement with previous works of Fashae *et al*. (2010), Ajayi and Egbebi. (2011); Ifeanyi *et al*. (2013), which reported high rates of*Salmonella*speciesmultiple antibiotic resistances to commonly used antimicrobial agents. The resistance may be associated with indiscriminate use of antibiotics by clinicians and poultry farmersas suggested by Kilozo – Nthenga *et al*.(2008). This study therefore presents the isolation of *Salmonella species* from apparently healthy chickens in poultry farms and slaughtered houses in Gwagwalada and Kwali areas of Abuja, suggesting a potential source of human Salmonellosis as 1cfu/g *Salmonella* isolates has been incriminated in food poisoning (Tambuwal *et al*, 2009). In conclusion this study provides preliminary reports on the burden and occurrence of *Salmonella* in chickens in the study area. Hence the need for regular testing as well-as formulation of a possible articulated vaccination control program is thus advocated. In addition, farmers should ensure strict hygienic practice in their farms and avoid indiscriminate use of antibiotics.

Acknowledgment

Authors wishes to acknowledge the staff of Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine University of Abuja for their effort towards the completion of this research work.

References

[1] Abdu, P. A. (2007). Fowl typhoid In: Manual of Important Poultry Disease in Nigeria 2nd Edition. Mac Chin Multimedia Designers, Zaria, Pp. 42-47.

[2] Agada, G.O., Abdullahi, I.O., Aminu, M., Odugbo, M., Chollom, S.C., Okeke, L.A and Okwori, A.E.J. (2014). Risk factors associated with Salmonella species contamination of commercial poultry farms in Jos Plateau State. International Journal of Current Research, 6: 6292-6301.

[3] Aghaje, M., Davies, R., Oyekunle, M. A., Ojo, O. E., Fasina, F. O. and Akinduti, P.A.(2010). Observation on the occurrence and transmission pattern of Salmonella gallinarum in commercial poultry farms in Ogun State, Southwest, Nigeria. African Journal of Micorobiological Research. 4 (9): 96- 800.

[4] Ajayi, A.O. and Egbebi, A. O. (2011). Antibiotic susceptibility of SalmonellaTyphi and Klebsiella pneumonia from poultry and local birds in Ado Ekiti, Ekiti State Nigeria. Annals of Biological Research, 2 (3): 431 – 437.

[5] Anon (2007). Gwagwalada and Kwali In: Federal Republic of Nigeria Official Gazette.Barrow, P.A. and Freitas, N.O.C. (2011). Pullorum and fowl typhoid disease-new thought on old diseases: A review. Avian Pathology, 40(1): 1-13.

[6] Bauer, A.W., Perry, D.M., Kirby, J.C. and Turck, M. (1966). Antibiotity sensitivity testing by a standardized single disk method. America Journal of Clinical Pathology, 36:493-496.

[7] Faruk, M., Tambuwal, A. S., Mikail, B., Abubakar, M. D., Salihu, M. H., Van Woudenbergh, C. J., Bokkers, E.G.M, de Boer, I.J.M. (2005). Risk factors of Salmonella Enteritidis infections in laying hens. PoultryScience, 84: 1308-1313.

[8] Fashae, K., Ogunsola, F., Aarestrip, F. M. and Hendriksen, R. S. (2010). Antimicrobial susceptibility and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. Journal of Infections in Developing Countries, 3; 4(8): 484 – 494.

[9] Harries, I. T., Fedorka, C. P., Gray, J.T., Thomas, L. A., Ferris, K. (1997). Prevalence of Salmonella organism in swine feed. Journal of American Veterinary Medicine Association, 201: 382- 385.

[10] Ifeanyi, C. I.C., Bassey, E. B., Ikeneche, N. F., Isu, R. N., and Akpa, A. C. (2013). Prevalence and Antimicrobial Susceptibility of Salmonella Specie Associated with Childhood Acute Gastroenteritis in Federal Capital Territory Abuja, Nigeria. British Microbiology Research Journal, 3: 2231- 0886.

[11] Jense, A.N. and Hoofar, J. (2003). Optimal purification and sensitivity quantification of DNA from fecal samples. Journal of Rapid Automatic Microbiology, 10: 231- 244.

[12] Kilozo, A., Nahashon, S. H., Clen, F. and Adefope. (2008). Avian Salmonellosis. Poultry Science. 87: 1841 – 1848.

[13] Nwachuckwu, E. and Nwiyi, P.O. (2011). Occurrence, effect and control of pathogenic Salmonella in some poultry farms in Abia State. Prime Journal of Microbiology Research, 3: 41- 46.
[14] National Committee for Clinical Laboratory Standards. (2004). "Performance standards for Antimicrobial susceptibility testing. Approved standard M100-S14."14th informational supplement.
[15] Mdegela, R.H., Yongolo, M.G.S., Minga, U.M. and Olsen, J.E. (2000). Molecular Epidemiology of Salmonella gallinarum in chickens in Tanzania. Journal of Avian Pathology. 29: 457- 463.
[16] Murugkar, H. V., Rahman, H., Kumar, A., Bhattacharyya, D. (2005). Isolation, Phage typing, and Antibiogram of Salmonella from man and animals in Northeast India. Indian Journal of Medicine, 122: 237-42.
[17] Mike, U.O., Henry, C. O., and Theodore, I. M. (2004). Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. In: Journal of Infectious Diseases 4: 16 – 18.
[18] Office Internatisonal des Epizooties (OIE). (2010). Fowl typhoid and pullorum diseases. In: Manual of Standards for Diagnostic Tests and Vaccines, 3rd Ed. OIE, Paris, Pp. 532-538.
[19] Office International Des Epizooties (OIE). (2005). Fowl typhoid In: Manual of Standards for Diagnostic Tests and Vaccines Standard, chapter 3.1.16.ap. 1-3 Institute for International cooperation in Animal Biologics.
[20] Okoli, I.C., Ndujihe, G.E., Obgbemu, I.P. (2006). Frequency of isolation of Salmonella from commercial poultry feeds and their antimicrobial resistance profile in Imo State, Nigeria.Journal of Health Allied Science, 5: 2 – 3.
[21] Parmar, D. and Davies, R. (2007). Fowl typhoid in a small backyard laying flock. Veterinary Record, 160: 348.
[22] Proux, K. , Humbert, F., Jouy, E., Houdayer, C., Lalande, F., Oger, A. and Salvat, G. (2002). Improvements required for the detection of S. Pullorum and S Gallinarum.Canadian Journal of Veterinary Research, 66, 151–157.
[23] Raufu, I., Hendriksen, R.S., Ameh, J. A., and Aarestrup, F. M. (2009). Occurrence and Characterization of Salmonella hiduddyify from chickens and poultry meat in Nigeria. Foodborne Pathology and Diseases, 6 (4): 425 – 430.
[24] Rao, D.G. (2000). A Comprehensive Textbook on poultry pathology, first Edition, published by RK Yadav limited, Pp 3-14.
[25] Shivaprasad, H. L. (2000). Fowl typhoid and pullorum disease. Reviews of Science and Technology, 19(2): 405-424.