ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *SALMONELLA* SPECIES FROM RAW CHICKEN MEAT SOLD IN JOS SOUTH L.G.A, PLATEAU STATE

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Abstract

This study aimed to determine the hygienic quality of raw chicken meat sold in Jos south LGA of Plateau State. A total of 200 chicken meats from different part and different slaughter points were collected and transported to microbiology laboratory for analysis. The overall prevalence of *salmonella* organisms was 46%. The prevalence in relation to location shows 27.2% (25/50), 22.8% (21/50), 20.7% (19/50) and 29.3% (27/50) from Gyel, Kuru, Du and Vwang district respectively. Prevalence of 4.3% (29/72), 45% (36/80) and 56.3% (27/48) were obtained from broiler, layers and indigenous type respectively. The neck and ventriculus recorded the highest prevalence of 78.3% (18/23) and 71.4% (15/21) with no prevalence recorded in thigh muscles. There was statistically significant association ($p< 0.05$) between parts of chicken and the prevalence of *salmonella* organisms in the study area. Ten antibiotics were tested on 15 (16.3%) of the isolates. Augmentin shows 100% susceptibility to all isolates. Ciprofloxacin, Septrin and Chloramphinicol shows 73% (11/15) each, while Erythromycin, Streptomycin Neomycin and Amoxicillin shows 46.7% (7/15) each. Ampicillin and Gentamycin shows the highest resistance of 40% (6/15) each. In conclusion, the results showed high prevalence of S*almonella* spp in chicken meat and high level of antimicrobial resistance pattern of the isolated salmonellae were observed.

Key words: chicken, *salmonella*, isolation, antibacterial susceptibility, Jos south

Introduction

Poultry keeping is a source of pleasure as well as income generation. It has become increasingly popular both in urban and rural areas (Gueye, 2003). Poultry meat is an important source of high quality proteins, a continuous source of income. It is not seasonal and can produce income for the entire year, poultry being available even among the poorer part of the population who cannot afford to keep pigs, cattle, sheep and goat (Ekunwe and Akahomen, 2015). Salmonellosis is an infection cause by *Salmonella* spp (Giessen *et al.,* 2006). *Salmonella spp* are gram negative, bacilli rod shaped bacteria, and they are mostly non-lactose fermenters, facultative anaerobic non spore forming belonging to the family *Enterobacteriaceae* (Moschonas *et al*., 2012). Salmonella is one of the most commonly reported causes of food- borne disease in European union and shows the highest disease burden on population scale among bacterial food-borne pathogens (Purushottam *et al.,* 2014). Contamination of meat with salmonella is still considered a major problem in food hygiene (Stock and Stolle 2001). Arguello *et al.,* (2013) stated that salmonella contamination in animal entering the slaughter house can be attributed to several

source such as holding pen, transport animal visceral and processing facilities.

Today there is little awareness about antibacterial resistance development in many countries worldwide not only among farmers but also among veterinarians and Government representative. Larger measured against resistance development are not prioritized in many countries even though it is listed by the UN as a measure of health issue (WHO1998) as more and more people get access to antibiotic for treatment of both human and animals the concern is great for a growing development of antibiotics resistance and other pathogens.

Although the demand for poultry meat is increasing day by day still this industry is facing some of the major problem like bacterial infections (Abiding *et al*., 2011). The incidents of the *Salmonella* have been reported in many countries EL-AZIZ, (2013). Thus the surveillance of antimicrobial resistance in zoonotic bacteria such as *Salmonella* is essential for providing information in the magnitudes and trends of resistance in food-borne pathogens in every country (WHO, 2001). Shahid *et al.,* (2009) stated that curing bacterial infection has determination of specific antibiotic for the treatment of salmonellosis.

The main aim of this study was to evaluate the hygienic quality of raw chicken meat slaughtered in Jos South Local Government Area of Plateau State, Nigeria with reference to isolation and antibiotic susceptibility profile of S*almonella spp* against commonly used antibiotics.

Materials and Methods

Study area

This study was carried out in Jos south Local Government Area of Plateau State, Nigeria. The Local Government has four districts namely: Vwang, Du, Kuru, and Gyel. The Local Government is located South of Jos North between the longitude 8^0 48W and latitude 9^0 94N in North central geopolitical zone of Nigeria the head quarter is in the town of Bukuru. It has an area 5110km^2 and a population of 306,761 at 2006 census (NIPOST 2009).

Sample collection

A total of 200 raw chicken meats were randomly collected from broilers, layers and indigenous type of chicken (50 samples from each district of Jos South Local Government Area) at different slaughter points. Different part of the chicken meat were collected and placed into sterile polythene bags and were taken in a cold flask to the Microbiology Laboratory of Federal College of Animal Health and Production Technology, Vom, for analysis.

Media preparation

Nutrient Agar

28g of nutrient powder was dispersed in 1 litter of sterile distilled water and allowed to dissolve for 10 minutes, swirled to mix the solution which was afterward autoclaved for 15minutes at 121^0C, then cold to 47^0C and poured into plates.

Peptone Water

15g of peptone powder was dissolved into 1litter of distilled water and was properly mixed and dispensed into McCartney sample bottles. They were then sterilized in autoclave for 15 minutes at 121^0C.

Salmonella Shigella Agar (SSA)

63g of the agar powder was dissolved into 1litter of sterile distilled water and mixed thoroughly. It was then heated with frequent agitation and boiled for a minute to completely dissolve the powder. Sterilization followed by autoclaving for 15 minutes at 121^0C and allowed to cool to 45^0 C. it was gently agitated before being poured into plates.

Sample Analysis

Bacteriological Examination

The freshly collected sample was homogenized using laboratory blender. 1g of the homogenized sample was placed into 9ml of peptone water and was incubated overnight at 37^0C for 24 hours for enrichment, and was followed by sub-culture on *Salmonella Shigella* and the plates were incubated at 37^0C for 24 hours. The plates were observed for colony formation after 24-48 hours of incubation where a typical black colored colonies surrounded by narrow green margin on the plate was an indication for *Salmonella* organism recommended for international organization for standardization (IOS) and previously described by Swayne *et al*., 1998.

Antimicrobial Susceptibility Test

For determination of susceptibility to antimicrobial agents, the disc diffusion method on nutrient agar was used. The isolates were sub cultured to nutrient agar. The following antimicrobial agents were tested: Ampicilliin (30µg), Augmentin (30µg), Streptomycin (10µg), Ciproflaxin (30µg), Septrin (20µg), Gentamycin (30µg), Chloramphenicol (20µg), Erythromycin (30µg), Neomycin (30µg) and Amoxicillin (30µg). Following the application of the antimicrobial discs, the plates were incubated at 37^0C for 24 hours. The diameters of the zones of inhibition were measured (millimeters) and were compared to internationally accepted measurements to determine the susceptibility or resistance of the isolate as described by Quinn *et al* (1994). Drug resistance patterns of the organisms were determined at three levels: susceptible (S), intermediate (I), and Resistance (R). The numbers of isolates of salmonella which showed S, I and R patterns were determined. The percentages of antimicrobial resistance of each pattern (S, I and R) of isolates were calculated and reported as the results.

Data Analysis

The data obtained were analyzed using chi-square method and p values < 0.05 were considered statistically significant. The results were presented in tables and expressed in simple percentages.

Results

A total of 200 chickens parts were obtained at slaughter in Jos south local government area of Plateau state. The prevalence of S*almonella spp* at slaughter, based on location is as shown in **Table I.** Out of 50 samples collected from each district, 25 (27.2%) from Gyel, 21 (22.8%) from Kuru, 19 (20.7%) from Du and 27 (29.3%) from Vwang were positive. There was statistically no significant association (χ^2 = 1.942, P > 0.05) between the districts and the prevalence of S*almonella* infection in the study area.

Table II shows the prevalence of *Salmonella spp* in relation to types of chickens. 72 samples were collected from broilers, 80 from layers and 48 from indigenous (Local) chicken. 29 (40.3%), 36 (45%) and 27 (56.3%) were positive for broilers, layers, and indigenous (local) respectively. There was no significant association (χ^2 = 1.079, P> 0.05) between the types of chicken and the prevalence of *salmonella* infection in the study area.

Table III shows the prevalence of *Salmonella spp* in relation to parts of chicken sampled. 29

liver samples were collected and 5 (17.2%) were positive. Out of 23 neck samples collected, 18 (78.3%) were positive, 15 (71.4%) out of 21 ventriculus samples were positive, 34 heart samples shows that 10 (29.4%) were positive, 17 wing muscles shows that 8 (47.1%) were positive, and 17(60.7%) out of 28, 19 (54.3%) out of 35 samples were positive from skin and ovaries respectively. There was no positive out of 22 samples collected from the thigh muscles. There was statistically significant association (χ^2 = 17.801, P < 0.05) between the parts of chickens and the prevalence of *salmonella spp* in the study area.

Table IV shows the susceptibility test of *Salmonella* isolates to commonly use anti-biotics. The isolates shows high resistance to Ampicillin and Gentamycin with 40% each, while Erythromycin, Septrin, Neomycin and Streptomycin shows less resistance with relatively average of 23.3%, Ciprofloxacin and Chloramphenicol shows less resistance of 6.7% each, with no resistance are Augmentin and Amoxicillin.

It was also observed that Ampicillin and Amoxillin shows th e highest intermediate with 53.3% each, Streptomycin, Chloramphenicol and Erythromycin shows less intermediate with 20% each. Augmentin shows the highest level of susceptibility with 100%, Ciprofloxacin, Septrin and Chloramphenicol also shows a very high susceptibility of 73.3% each. Streptomycin, Neomycin and Amoxicillin shows 46.7% level of susceptibility each. Ampicillin shows less susceptibility with 6.7%

Table I: Prevalence of *salmonella spp* based on location

Location	number of sample	positive	percentage
Gyel	50	25	27.2%
Kuru	50	21	22.8
Vwang	50	27	29.3%
Total	200	92	100

χ^2 = 1.942 df = 3 P. value = 0.75439

TABLE 2: Prevalence of *Salmonella* spp in relation to type chicken

Type of chicken	No of samples	Positive	Percentage (%)
Broiler	72	29	40.3
Layers	80	36	45
Indigenous	48	27	56.3
Total	200	92	100

χ^2 = 1.079, df = 2, P = 0.58304.

Table 3: Prevalence of *Salmonella* spp based on chicken parts.

Part	No of sample	positive	Percentage (%)
Liver	29	5	7.2
Neck	23	18	78.3
Gizzard	21	15	71.4
Heart	34	10	29.4
Wing muscle	17	8	47.1
Skin	28	17	60.0
Ovaries	35	19	64.3
Tight muscle	13	0	0
Total	200	92	100

$\chi 2 = 17.801$ df = 7 p = 0.012898

Table 4: susceptibility test of *Salmonella* isolate to anti-biotics

Antibiotics	Disc potency (µg)	Resistance No (%)	Intermediate No (%)	SusceptibilityNo (%)
Amp.	30	6 (40)	8 (53.3)	1 (6.7)
Aug.	30	0 (0)	0 (0)	15 (100)
Strep.	10	3 (20)	5 (33.3)	7 (46.7)
Cipro.	30	1 (6.7)	3 (20)	11 (73.3)
Sep.	20	4 (26.7)	0 (0)	11 (73.3)
Gen.	30	6 (40)	5 (33.3)	4 (26.7)
Chloram.	20	1 (6.7)	3 (20)	11 (73.3)
Erythro.	30	4 (26.7)	3 (20)	8 (53.3)
Neo.	30	3 (20)	5 (33.3)	7 (46.7)
Amox.	30	0 (0)	8 (53.3)	7 (46.7)
Total				

$\chi^2 = 0.3033$, df = 9, P = 0.002413.

Discussion

The food borne Disease Active surveillance Net-work (Food Net) of the United States centers for Disease Control and Prevention reported that incidence of *salmonella* infection was highest among the total food poisoning cases (CDC, 2010). The present study shows the overall prevalence of 46% S*almonella* spp in chicken slaughtered in Jos South Local government Area of Plateau State. The high prevalence recorded in this study agrees with previous report: 42.3% in Korea (Hyeon *et al.,* 2011),72% in Thailand (Angkititrakul *et al*., 2005), 35.5 to 47.7% in Austria (Pointon *et al*., 2008), 52% in China (Yang *et al.,* 2011),

and 53% in Vietnam (Van *et al.,* 2007), but in contrast to the findings of Jethro, (2013) who recorded a lower prevalence of 11% in Jos, Nigeria and 22.4% in Korea (Kim *et al*., 2012). We speculate that the difference of *salmonella* prevalence between reports might be associated with difference in hygiene and sanitation levels of each country and the different methods used in each study. It could also be due to variations in season of the research.

The study also revealed that samples collected from the neck and ventriculus part of the chicken recorded the highest prevalence of 78.3% and71.4% respectively. This is not in agreement with the report of Edel, (1994) who reported a lower prevalence of 36% and 23%. This might be attributed to unhygienic post slaughtered operations and direct contact of the meat with fecal content as stated by Nauta *et al*., (2013).

Among the antibiotics used in this study, the highest resistant was observed against ampicillin (40%) and gentamycin (40%) followed by erythromycin, neomycin and streptomycin at an average of 23% each. the high prevalence of resistance could be related to uncontrolled use of these antimicrobial agents in the treatment of bacterial infection. Besides, the highest sensitivity was found to augmentin, septrin, chloramphenicol and ciprofloxacin. The high sensitivity of the isolated *salmonella* to the mentioned antibiotics could be related to less frequent usage of these drugs for therapeutic purposes, therefore reducing the chance of resistance to develop. These findings are in agreement with Fashae *et al* (2010), Gordana *et al* (2012), Jethro, (2013), Jahantigh *et al* (2015), Tamba *et al* (2016) reported similar susceptibility in chloramphenicol and ciprofloxacin and septrin in similar work in different part of Nigeria and other parts of the world.

Conclusion

This study has revealed high prevalence of *Salmonella* spp in chicken meats sold in Jos south local government of plateau state. We have further discovered that most of the isolates were resistant to most commonly used antibiotics for treatment of infections. As animals are the reservoir of salmonella and the use of antimicrobials in food animals for therapy, prophylaxis and growth promotions accelerate the emergence of antimicrobial resistant pathogens, it is not surprising that an increased number of human Salmonellosis cases are caused by food borne antimicrobial resistant *salmonella* (Foley and Lynne, 2008). According to the result of this study, chickens may have an important role to dispread *salmonella* in the environment.

Recommendations

Effort is needed to control salmonellosis in poultry flocks to reduce the threat of this organism for public health. Serious environmental hygiene and strict infection control measures must be adopted both in the treatment and disposal of animal drugs and at abattoirs to prevent the contamination of the food chain. The public should be properly enlightened on the dangers of eating raw or insufficiently cooked meat to forestall acquisition of these pathogens. Further studies are required to identify the particular strains of *salmonella* harboured by the meat and the level of risks they pose to the public.

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References

Abiding, Z., Khatoon, A. and Numan, M. (2011). Mycotoxins in broilers: pathological alterations induced by aflotoxins and oxchratexins, diagnosis and dertermiination, treatment and control of mycotoxicosis. *World poultry farm Journal, 57: 485-496.*

Angkititrakul, S., Chomvarin, C., Chaita, T., Kanistanon, K. and Waethewutajarn, S. (2005). Epidemiology of antimicrobial resistance in salmonella isolated from pork, chicken meat and humans in Thialand. *The Southeast Asian Journal of Tropical Medical and Public Health*. 36.1510-5.

Arguello, H., Carvajal, A., Naharro, G., Arcos, M., Rodicio, M.R., Martin, M.C. and Rubio, P. (2013). Sero-and genotyping of *Salmonella* in slaughter pigs, from farm to cutting plant, with a focus on the slaughter process. *International Journal of Food Microbiology*.161 (1): 44-52.

CDC. (2010). Number of laboratory-confirmed bacterial and parasitic infections, by year andpathogen. Food-borne Diseases Active Surveillance Network (*FoodNet*), United States, 1996–2009.

Edel, W. (1994). *Salmonella enteritidis* eradication program in poultry breeder flocks in Netherlands. *International Journal of food Microbiology,* 21:717-718.

Ekunwe, P. A. and Akahomen, A. (2015). Assessment of the Profitability of Broiler Production In Edo State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment.* 11(4):6-11.

El-Aziz, D.M.A. (2013). Detection of *salmonella typhimurium* in retail chicken meat and chicken giblets. *Asian Pacific Journal of Tropical Biomedicine;* 3: 678-681.

Fashae, K., Ogunsola, F., Aarestrup, F.M. and Hendriksen, R.S. (2010). Antimicrobial susceptibility and serovars of salmonella from chickens and humans in Ibadan, Nigeria. *Journal of Infection in Developing Countries*. 4(8):484-494.

Foley, S L. and Lynne, A M. (2008) Food Animal Associated Salmonella Challenges: Pathogenicity and Antimicrobial Resistance. *Journal of Animal Science*, 86, E173-E18.

Giessen van de, A.W., Bouwknegt, M., Dam-Deisz, W.D.C. and van Pelt, W. (2006). Surveillance of *Salmonella* spp. and *Campylobacter* spp. in poultry production flocks in The Netherlands *Epidemiological Infection*, 134, 1266–1275.

Gordana, M., Bogdanka, A., Dragica, T., Milena, L. and Brankica, D. (2012). Antibiotic Susceptibility of *Salmonella spp:* A comparison of two surveys with a 5 years interval. *J. Imab-annual Proceeding* (scientific papers). 18(1):216-219.

Guèye, E.F. (2003). Poverty alleviation, food security and the well-being of the human population through family poultry in low-income food-deficit countries. *Journal of Food, Agriculture Environment,* vol 1(2): 12–21.

Hyeon, J. Y., Chon, J.W., Hwang, I.G., Kwak, H.S., Kim, M.S., Kim, K.S., Choi, I.S.,Song,C.S., Park, C. and Seo, K.H. (2011). Prevalence, antibiotic resistance, and molecular characterization of Salmonella serovars in retail meat products. *Journal of Food Protection*. 74:161–166.

Jahantigh, M., Jafari, S.M., Rashki, A. and Salari, S. (2015) Prevalence and Antibiotic Resistance of Salmonella spp. in Turkey. *Open Journal of Medical Microbiology*, 5, 113-117.

Jethro, D. (2013). Isolation and antibiotic sensitivity patterns of Salmonella species in raw beef in Jos South Local Government Area of Plateau State, Nigeria. HND project submitted to Federal College of Animal Health and Production Technology, Vom, Plateau State. Pp 21.

Kim, M.S., Lim, T.H., Jang, J.H., Lee, D.H. and Kim, B.Y. (2012). Prevalence and antibiotic resistance of *Salmonella* species isolated from chicken meats produced by different integrated broiler operations in Korea. *Poultry science*. 91:2370-2375.

Moschonas, G., Geornaras, I., Stopforth, J.D., Wach, D., Woerner, D.R., Belk, K.E., Smith, G.C. and Sofos, J.N. (2012). Antimicrobials for reduction of Salmonella contamination in uncooked, surface-browned breaded chicken products. *Journal of Food Protection*. 75(6): 1023-1028.

Nauta, M., Barfod, K., Hald, T., Sørensen, A. H., Emborg, H.D. and Aabo, S. (2013). Prediction of Salmonella carcass contamination by a comparative quantitative analysis of E. coli and Salmonella during pig slaughter. *International Journal of Food Microbiology*. 166(2): 231-237.

NIPOST. (2009). "Post Offices with Map of Local Government Area". Archived from the original on 2009-10-07.

Pointon, A., Sexton, M., Dowsett, P., Saputra, T., Kiermeier, A., Lorimer, M., Holds, G., Arnold,G., Davos, D., Combs, B., Fabians-son, S., Raven, G., McKenzie, H., Chapman, A. and Sumner, S. (2008). A baseline survey of the microbiological quality of chicken portions and carcasses at retail in two Australian states (2005 to 2006). *Journal of Food Protection*. 71:1123–1134.

Purushottam K., Anjay, Savita K., Sanjay, K.B. and Shanker, D. (2014). Isolation and prevalence of *Salmonella* from chicken meat and cattle milk collected from local markets of Patna, *Veterinary World,* EISSN: 2231-0916.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (1994). Clinical Veterinary Microbiology. Wolf Publishing, London. Pp 209-236.

Shahid, M., Farrukh, S. and Anuradha, S. (2009). AMPL B- lactamases and bacterial resistance: an update mini review. *Reviews in Medical microbiology.* 20 (3): 41-52.

Stock, K. and Stolle, A. (2001). Incidence of Salmonella in minced meat produced in a European Union-approved cuttisng plant. *Journal of Food Protection*. 64(9): 1435-1438

Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E. and Reed, W.M. (1998). A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th Edition, American Association of Avian Pathologists, University of Pennsylvania, Kennett Square.

Tamba, Z., Bello, M. and Raji, M.A. (2016). Occurance and antibiogram of salmonella Spp. In raw and fermented milk in Zaria and Environs. *Bangladesh Journal of Veterinary Medicine*. 14(1): 103-107.

Van, T. T., Moutafis, G., Istivan, T., Tran, L.T and Coloe, P.G. (2007). Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Applied Environmental Microbiology*. 73:6885–6890.

WHO, (2001) (Global Strategy for containment of Antimicrobial resistance) WHO, Geneva. *WHO/cDc/ CSR/DRS/2001.2.*

World Health Organization (1998). Emerging and other communicable Disease, surverllance and control, use of Quinolines in Food Animal and potential impact on Human Health: report of a WHO meeting Geneva, Switzerland 2-5 June, *WHO/EMC/ZDI/98.10.*

Yang, B., Xi, M., Wang, X., Cui, T., Yue, T., Hao, H., Wang, Y., Cui, Y., Alali, W.Q., Meng, J.,Walls, I., Wong, D.M. and Doyle, M.P. (2011). Prevalence of *Salmonella* on raw poultry at retail markets in China. *Journal of Food Protection*. 74:1724–1728.