Original Research

Isolation, Identification and Antimicrobial Susceptibility Profile of *Salmonella* spp. from Poultry Farms in Jos South Local Government Area of Plateau State, Nigeria

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Abstract

The study was carried out to biochemically determine the presence of salmonella spp., in poultry farms, detect the presence of 16S DNA using conventional polymerase chain reaction and to know the antimicrobial susceptibility profile of the isolates circulating in poultry farms in Jos south local government area of Plateau state, Nigeria. Grams staining culture and biochemical property identified 9(39%) as Salmonella spp. where 7(2.1%) were identified as Salmonella gallianarum by being non-motile and 2(0.6%) were the other motile Salmonellae and 5(6.0%) samples generated amplification products of 284 bp after the PCR with specific primers which targeted invA gene associated with Salmonella spp. Most of the isolates were resistant to the drugs tested. Five of the isolates were susceptible to Ciprofloxacin, two isolates were susceptible to Chloramphenicol and Neomycin and only one isolate was susceptible to Sulphamethoxazole/Trimethoprim. This study highlighted the occurrence of resistance to important antimicrobials like amoxicillin and erythromycin. As antimicrobials are easily obtainable over the counter, there is need for veterinary authorities in the state to regulate antimicrobial use in poultry farms and in clinics to limit the spread of multidrug-resistant salmonella among poultry farms.

Key words: Antimicrobial Susceptibility, Isolation, Molecular Characterization, *Salmonella* spp.

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Introduction

Salmonella species have been considered as one of the most important foodborne pathogens around the world (Gillespie *et al*., 2003). Salmonellae are gram negative, non-lactose fermenting and non-sporing bacteria. With exception of *S. pullorum* and *S. gallinarum*, all salmonellae are actively motile (Cheesbrough *et al*., 2000). *Salmonella* are widely distributed in nature and survive well in a variety of foods and contamination can occur at multiple steps along the food chain (Pui *et al*., 2011). Many epidemiological studies have reported the wide variety of routes by which *Salmonella* can be disseminated within integrated poultry farms and across geographical areas in different countries at different times (Norgrady *et al*., 2003; Namata *et al*., 2008). Infection with *Salmonella* can occur through inadequate cleaning and disinfection of poultry houses, presence of contaminated carriers especially rodents and insects, litters, water, dust, equipment and feed (Carrique-Mas *et al*., 2008; Marin & Lainez, 2009). Infection in day-old chicks could be vertical from infected breeder flocks or horizontally transmitted during hatching, loading and transporting to the farm (Chriel *et al*., 1999).

In 2011, Nigerian hen egg production totaled 636,000 metric tons (MT) and was valued at $527.49 million, ranking 19th in world hen egg production and the top producer in Africa (FAO, 2015). Both large and small egg farms are scattered all over the country, although they are generally concentrated around the major urban centres (FAO, 2008). Poultry meat is one of the major sources of animal protein in Nigeria like in many developing countries because of its affordability and acceptability (Bettridge *et al*., 2014). This source of protein is however being threatened by diseases such as salmonellosis and avian influenza (FAO, 2006). Poultry salmonellosis remains a major constraint to poultry production in all parts of Nigeria (Mbuko *et al*., 2009). Farmers still experience great losses by way of mortality, morbidity and drop in egg production due to *Salmonella* infection despite huge amounts spent on vaccination and medication. Identification of *Salmonella* spp. can be performed *via* both serotyping and molecular methods. Serotyping offers a reliable method for differentiating *Salmonella* strains, but this procedure is time-consuming. However, molecular methods are fast, as well as highly sensitive and very specific (Mbuko *et al*., 2009).

This study was prepossessed to assess the distribution of *Salmonella* spp, and their susceptibility to most commonly used antibiotics in Jos South poultry farms.

Materials and Methods

Study Area

The study was conducted in Jos South Local Government Area of Plateau State, Nigeria. Jos South with it's headquarter at Bukuru 9^0 4800"N 8^0 52' 00" E. It has an area of 510 m^2 and a population of 306, 716 as at the 2006 census. The study covered four districts namely Du, Kuru, Vwang and Gyel. The major occupation in this area is crop and livestock farming. A total of 335 fecal samples consisting of 225 cloacal swab samples and 110 fresh droppings were collected aseptically into buffered peptone water on farm. The samples were transported to the bacterial research laboratory National Veterinary Research Institute (N.V. R. I) Vom on ice within 24 hours of collection for culture and isolation.

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Isolation and Identification of *Salmonella*

The isolation of *Salmonella* spp. was carried out using the method described by (Quinn *et al*., 2002). The samples were enriched in Rappaport-Vasilliadis broth. The fecal samples were inoculated into Rappaport-Vasilliadis broth and incubated at 37^0C for 24 hours. A loopful of the inoculated Rapport-Vasilliadis broth was streaked onto Xylose Lysine Desoxycholate (XLD) agar and incubated for 24 hours at 37^0C. The colonies were examined for the characteristic pinkish colour of *Salmonella* with or without hydrogen sulphide. Suspected colonies were then subjected to indole, motility, oxidase, urease, citrate, triple sugar iron (TSI) and sugar fermentation tests for identification of *Salmonella* spp.

Polymerase Chain Reaction PCR

Bacterial DNA was extracted using DNA extraction kit (Genaid, Korea) as described by the manufacturer. Conditions of PCR for *rfbsg* genes amplification of *Salmonella* was done according to (Paiva *et al*., 2009). All the isolates were tested by the conventional PCR targeting *invA genes*, as previously described (Kang *et al*., 2011). Amplification reaction was carried out in a 50 µl PCR mixture containing 5 µl of 10 x PCR buffer – Buffer Taq Gold (5 µM Tris-HCL, 50 µM KCl, pH 8.8), 4 µl of 2 µM MgCl^2, 1 µl of 200 µM dNTPs, 6 µl each of 0.6 *invA* forward and reverse primers; 4 µl each of 0.4 µM *speC* forward and reverse primers respectively; 0.3 µl of 1.5 U Tag Gold DNA Polymerase, 5 µl genomic DNA and 14.7 µl of demineralized water. The PCR was carried out using the following thermal profile: initial denaturation of 5 minutes at 95 ^{0}C followed by 30 cycles, each at 95 ^{0}C for 30 seconds, 60 ^{0}C for 30 seconds, 72^0C for 30 seconds, with a final extension step at 72 ^{0}C for 5 minutes. The electrophoresis was carried out using 1.5% agarose gel, and then stained with 1.5% ethidium bromide solution and fragments were viewed by UV transillumination.

Table 1: Oligonucleotide primers for conventional PCR for *salmonella*

Primer	Sequence (5' → 3')	Product (bp)
invA gene F	5' GTG AAA TTA TCG CCA CGT TCG GGC AA-3'	284 bP
R	5' TCA TCG CAC CGT CAA AGG AAC C-3	

Drug Sensitivity Pattern of *Salmonella* Isolates

The disc diffusion method was used to determine the drug sensitivity pattern of *Salmonella* isolates to seven different antimicrobial agents including; Streptomycin (30 µg), Amoxicillin (30µg), Chloramphenicol (30µg), Ciprofloxacin 5µg, Neomye/Trimethoprim 25µg and Erythromycin (5 µg) obtained from Fondiscs®. The *Salmonella* isolates were subcultured into Mueller-Hinton broth (Oxoid-CM0405) and then incubated at 37°C for 24 hours. Serial dilution was performed in the ratio of 2:4 of the broth and distilled water to reduce the degree of the turbidity of the broth culture. Then 1 ml was dispensed onto Mueller-Hinton agar and the excess discarded. Antibiotic discs were applied aseptically to the surface of the plate

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with the help of sterile forceps. This was incubated at 37ºC for 24 hours under aerobic condition to observe for zones of inhibition, which were measured with the aid of a ruler (Mondal *et al.*, 2008; Kaushik *et al.*, 2014).

Result

Out of the 335 sample collected 23(6.9) presumptive positive on XLD, 9(39) with biochemical test and 5(56) confirmed with PCR (Table 2).

Table 2: Different methods of isolation and identification of *Salmonella* spp from four districts in Jos south LGA

District	No. of samples tested	Positive on XLD	Percentage (%)	Positive Biochemical test	Percentage (%)	Positive PCR	Percentage (%)
Kuru	83	7	8.4	2	28.6	2	100
Vwang	83	6	7.2	3	50	1	33.3
Du	83	6	7.2	2	33.3	1	50
Gyel	83	4	6.3	2	50	1	50
Total	335	23	6.9	9	39	5	56

Table 3: Biochemical activities of different isolates the faecal samples obtained from four districts in Jos South LGA

Carbohydrate Fermentation Test						Indole	MR	VP test	Motility test	Dulcitol	Name of Isolated Bacteria
Isolates	**D**	**Xy**	**S**	**L**	**Mn**						
L2	AG	AG	-	-	AG	-	+	-	+	AG	OMS
L4	AG	AG	-	-	AG	-	+	-	-	-	*S. gal/S. Pul*
N2	AG	AG	-	-	AG	-	+	-	+	AG	OMS
L28	AG	AG	-	-	AG	-	+	-	-	AG	*S. gal/S. Pul*
L10	AG	AG	-	-	AG	-	+	-	-	AG	*S. gal/S. Pul*
C22	AG	AG	-	-	AG	-	+	-	-	-	*S. gal/S. Pul*
L35	AG	AG	-	-	AG	-	+	-	-	-	*S. gal/S. Pul*
L5	AG	AG	-	-	AG	-	+	-	-	-	*S. gal/S. Pul*
L2	AG	AG	-	-	AG	-	+	-	-	AG	*S. gal/S. Pul*

=Dextrose; Xy= Xylose; S= Sucrose; L= Lactose; Mn= Mannitol; MR= Methyl Red; VP= Vogues-proskauer test; AG= Acid and Gas, OMS= other motile Salmonella; + = Positive; - = Negative

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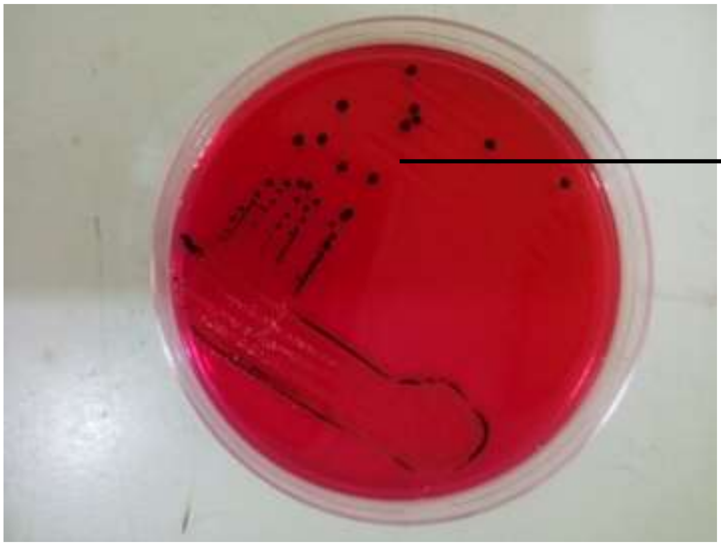

Plate 1: *Salmonella* isolates on XLD appearing as Pink-red colonies with black centers

Plate 2: *Salmonella* on TSI produces acid in the butt (yellowish color) while in the slant it produces alkaline (reddish color). There is also blackish coloration as a result of H_2S reaction. Gas production was also noticed in some of the species.

Results of Antimicrobial Susceptibility Test of *Salmonella* Isolates

The sensitivity and resistance patterns of nine salmonella isolates were to seven different antibiotic agents as shown in Table 4. Amoxicillin and Erythromycin had the highest resistance, 9(100.00%) to *Salmonella* isolates, followed by Streptomycins and Sulphamethaxazole/Trimethoprim 8(88.9.00%). Ciprofloxacin 5(55.6%) was susceptible to isolates. Chloramphenical7 (77.8%) and neomycin 6(66.7) were resistant to salmonella isolates (Table 4).

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Table 4: Antibiogram of *Salmonella* spp

Isolate	Amoxycillin AMC (30 µg)	Streptomycin S (10 µg)	Erythromycin E (5µg)	Ciprofloxacin CIP (5 µg)	Chloramphenicol C (30 µg)	Neomycin (10 µg)	Sulphamethaxazole/ Trimethoprim (25 µg)
C22	R	S 8 mm	R	S 20 mm	R	S 10 mm	R
S. Kentur	R	R		S 25 mm	R	S 11 mm	S 2 mm
L35	R	R	R	S 21 mm	R	R	R
L2 22 weeks	R	R	R	S 16 mm	R	R	R
L4 22 weeks	R	R	R	R	R	R	R
L4	R	R	R	R	S 13 mm	R	R
N2D11	R	R	R	R	R	R	R
L28	R	R	R	S 21 mm	S21mm	R	R
L10 22WKS	R	R	R	R	R		R

C22 = Chaha, S. kentu = Vom, L35 = layer35 wkss, L2 = layers2wks, l422 = layers 4 22 wks, N2D11=Anguldi broilers, L28 = layers 28wks, L10 = layers 10 wks.

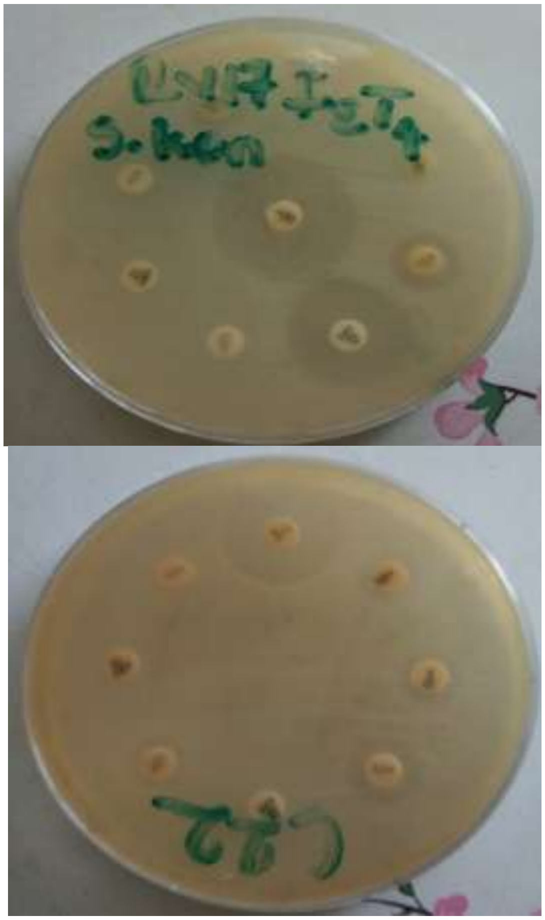

Plate 3: Culture of s*almonella* Species showing zones of inhibition and resistance on Muller Hinton agar

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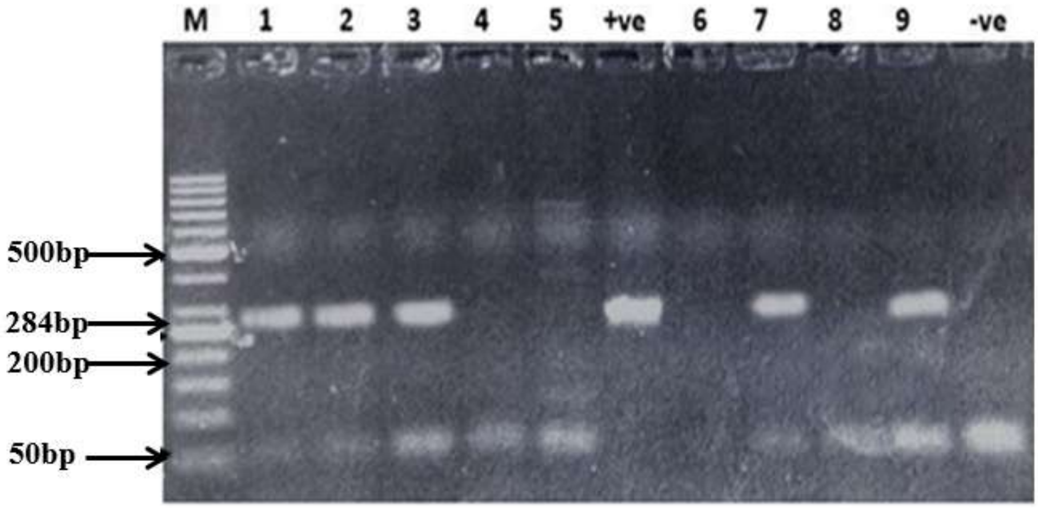

Fig. 1: PCR amplification mixture was run on 1.5% agarose gel stained with ethidium bromide. Lanes M, (1500 bp) Molecular weight marker; lanes 1- L10 isolate, L2- L2 Isolate, Lane 3- C22 isolate, Lane 4- L4 isolate, Lane 5-L28 isolate, Lane +ve - Rv17 FET4 positive control for invA gene (284bp), Lane 6-L35 isolate, Lane 7-N2D11B isolate, Lane 8-L4 isolate, lane 9- L21 isolate, Lane –ve- negative.

Discussion

In the present study, specific biochemical media were used for the detection of *Salmonella*. All of the isolates fermented dextrose, mannitol and xylose but did not ferment lactose and sucrose and all of the isolates were indole negative, methyl red positive and VP negative which are special biochemical characters for *Salmonella* spp (Table 1). Similar findings were previously documented by other scientists (Christensen *et al.*, 1993). In Gram's staining, the morphology of the isolated bacteria was small rod shape, gram negative, single or paired in arrangement which also corresponded with morphological characters of *Salmonella* as described by several authors (Freeman, 1995). In the present study seven of the isolates were non-motile and two were motile. Motility test was a fundamental basis for the identification of motile and non-motile *Salmonella* organisms anywhere they are found (Freeman, 1995). Non-motile organisms were considered to be either *S. Pullorum* or *S. Gallinarum*. The motile organisms were considered as others species of *Salmonella* under Paratyphoid group (Christensen *et al.*, 1993 & OIE manual, 1996). In the present study three out of seven isolated nonmotile salmonellae fermented dulcitol. On the basis of this dulcitol fermentation test, these three dulcitol fermenter can be grouped into *S. Gallinarum* (OIE Manual, 2010).

The results of this study indicated that the total number of *Salmonella* spp. identified by conventional techniques was 9(10.8) (Table 2), with the highest value revealed by conventional biochemical test in Vwang district with 3(3.6). Out of the nine isolates, five were further confirmed by PCR as *Salmonella* species. At Kuru district 2(2.4) were identified by both biochemical test and PCR which is the highest in the four districts examined. At Du, Vwang and Gyel districts a very low number of isolates were confirmed

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by the PCR method, 1(1.2). Pathogenic organism such as *Salmonella* has been a major concern to the public all over the world. Fowl typhoid is a poultry disease that has decreased in incidence over the years by application of basic management procedures which is applicable to Nigeria (Saif *et al*., 2003). In Nigeria, especially in many parts of Plateau state, poultry droppings serve as a good source of manure for the cultivation of crops and vegetables (Orji *et al*., 2005). The farmers use poultry droppings for the dual purposes of enriching the soil for improved crop yields and economically disposing of the droppings. However, the addition of the poultry droppings directly into soils without any form of treatment poses some public health problems since they contain pathogenic microorganisms. Poor sanitary habits have been reported to be contributing to the persistence of the disease (Jordan & Pattison, 1999) and have led to enteric disease endemicity. Sanitary awareness has been found below average from the findings of this study while interacting with the farmers in the study area. This is similar to the findings of Orji *et al.* 2005 in Awka, Anambra State, Nigeria. The pathogenic microorganisms can contaminate the surrounding crops and vegetables and become a source of infection, especially when such crops or vegetables are eaten raw or brought home where they can contaminate other materials.

Irrespective of the low prevalence, it should be regarded as significant owing to its devastating effect on infected birds and the poultry industry at large. Fowl typhoid is transmitted both vertically and horizontally. In cases where the disease is transmitted horizontally, contaminated feed and drinking water are the main sources of infection and, under extensive systems of management, as in village poultry production, the chances of chickens eating and drinking contaminated materials are minimal. Apparently, the infection rate can vary considerably with time, but the reason for this remains unknown. The possibility that the serological positivity was due to infection with other Group D *Salmonella*, such *as Salmonella Enteritidis* or *Salmonella Pullorum* cannot be excluded. It is possible that, under poor biosecurity systems as encountered in this study, commercial chickens may act as one of the sources of *Salmonella* for scavenging local chickens. Poultry droppings have been known to be constant sources of spreading poultry diseases. Previous studies performed in Tanzania and Senegal have reported higher serological prevalences in commercial layers than those observed in Nigeria (Orji *et al*., 2005). Generally, *Salmonella* infected chickens are not efficient excretors, unlike birds infected with motile *Salmonella* that cause enteric rather than systemic infections (Smith & Tucker, 1980). Owing to the indiscriminate use of antibiotics, many pathogens have developed resistance to antibiotics. The continued use of these pathogen resistant drugs could lead to development of more and highly resistant strains of the pathogen which can spread in the environment causing major disease outbreaks of an enormous magnitude in future.

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Conclusion

This study has shown that there is presence of *Salmonella spp* in poultry dropping in the study area. The present work demonstrated that all (100%) of the isolates were resistant to amoxycillin and erythromycin. Factors found to be responsible for *Salmonella* infection on farm contamination were of type of housing, farm biosecurity, feed and water management while fencing farm and use of protective clothing was found to reduce the risk of *Salmonella.* These poultry wastes could pose a health hazards to humans and other uninfected birds especially free-range birds. Further studies on the antibiotic susceptibility profile of bacterial pathogens of poultry origin are highly recommended.

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