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Molecular Detection and Antimicrobial Susceptibility of Salmonella Species in Chickens

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A B S T R A C T

The study was intended for molecular detection of Salmonella Spp isolated from chicken. A total of 160 samples comprising of 40 liver, 40 spleen, 40 lungs and 40 intestines were collected directly into buffered peptone in universal bottles at the poultry slaughter houses of the four districts in Jos South Local Government area of Plateau State. The samples were enriched in 10 ml of Rappaport-Vassiliades broth and cultured onto Xylems Lysine Deoxycholate (XLD) agar for the isolation of bacteria. The isolated bacteria were identified by studying staining characteristics, cultural properties on different selective media, biochemical tests, catalase and coagulase test, and finally by PCR. Out of 160 samples, 65 (41%) samples were found positive for Salmonella Spp on XLD and 24 (37%) positive by biochemical analysis. Two*(*2) Salmonella isolates were amplified by 942 bp gene based PCR. Antimicrobial sensitivity test was carried out to ascertain the susceptibility of the organism to various antibiotics. Its results showed that the Salmonella *isolates* were resistant to amoxycillin (100%) and erythromycin (100%), gentamicin (100%), Clindamycin (100%) streptomycin (100%), tetracycline (100%), sulphamethoxazole/trimethoprim (100%) but sensitive to Ceftiofur (100%).

Keywords

Molecular detection, Antimicrobial Susceptibility, Salmonella in Chicken

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Introduction

Salmonellae are Gram-negative facultative anaerobic rod-shaped bacteria that measure 0.7-1.5 by 2.0-5.0 µm, non-sporogenic. *Salmonella* species have been considered as one of the most important foodborne pathogens around the world. *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.,* 2016).

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.,* 2014). 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). 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.,* 2017).

In 2011, Nigerian hen egg production totalled 636,000 metric tons (MT) and was valued at 8527.49 million, ranking 19th in world hen egg production and the stop producer in Africa (FAO, 2012). Both large and small egg farms are scattered all over the country, although they are generally concentrated around the major urban centres (FAO, 2012). 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 *salmonellasis* and avian influenza (FAO, 2012). Poultry *salmonellosis* remains a major constrain to poultry production in all part of Nigeria. The aim of this study was to detect and characterize *Salmonella* spp. in commercial chicken in Jos South Local Government area of Plateau state.

Materials and Methods

Study location

The study was conducted in Jos-South Local Government area of Plateau State Nigeria. Jos-South with its headquarter at Bukuru 9^0 4800" N $8^0$5200" E. It has an area of 510m^2 and population of 306,716 as at the 2006 census (Rim, 1993). The study covered Kuru, Vwang, Kugiya and Gyel.

Sample collection

A total of 160 samples comprising of 40 liver, 40 spleen, 40 lungs and 40 intestines were collected directly into buffered peptone in universal bottles at the poultry slaughter houses of the four districts in Jos South Local Government area of Plateau State. All samples were transported on ice to Salmonella laboratory bacterial research for analysis.

Isolation of Salmonella

Bacterial culture and identification were carried out in the Salmonella laboratory National Veterinary Research Institute Vom. Tissue samples of the organs liver, spleen, lungs and intestine were enriched in 10 ml of Rappaport-Vassiliades broth (Oxoid Basingstoke, UK)). Following the enrichment, samples were incubated at 37°Cfor 24 hours. Using a sterile wire loop, a loopful of each incubated broth culture were inoculated onto Xylose Lysine Deoxycholate (XLD) agar (Oxiod, Basingstoke, UK) and incubated at 37°C for 24 hours. The plates were examined for typical colonies of *Salmonella.* The colonies from the plates were further subcultured on XLD agar and incubated at 37°C for 24 hours. The plates from the sub cultured plates were observed for typical colonies of *Salmonella* as described suspected colonies were subjected to, urease, and triple sugar iron TSI Doughlas *et al.,* (1998).

Phenotypic Identification of Salmonella

Pure colonies of *Salomonella* were picked up from XLD plate with bacteriological loop, emulsified with a drop of distilled water on a clean greasy-free slide and fixed by gentle

heating. Crystal violet was applied on each smear to stain for 1 minute and then wash with water. Then Lugols iodine was applied for 1 minute and again wash with water.

This was followed by addition of 95% alcohol which served as decolourizer and allowed to stand for a period of 10 seconds. After rinsing with water, safranin was used to counter stain and then washed with water after 30 seconds.

The preparation was air dried and examine under the microscope with high power objective (X100) using oil immersion Doughlas *et al.,* (1998).

Molecular detection of Salmonella

A set of primer pair specific for the *invA*gene for *Salmonella* species with sequences base pair 942bp was used (Galán *et al.,* 1992). Primer sequences: invAf5-3[1]- GTG AAAT TATC GCCA CGTTCGGGCAA and invAr5–3[1]– TCA TCGC ACCG TCAA AGGAACC.

Antibiotic susceptibility test of Salmonella isolated from commercial chickens

All the isolates were tested for antibiotic susceptibility using Kirby-Bauer *et al.,* 1966 diffusion assay. The antibiotic be tested includes, Tetracycline 30µg, Amoxycillin 25µ, sterptomycine 10 µg, sulphamethoxazole trimethoprime 25 µg, Ceftiofur 30 µg.

The inhibition zone around each was measured independently and compared with standard interpretive charts: Clinical Laboratory Standard Institute (CLSI, 2006). Zone size for each antimicrobial agent was measured independently before comparison.

Results and Discussion

Isolation of Salmonella from various locations in the study area

From a total of 160 samples subjected to bacteriological examination on XLD. The distribution of Salmonella isolated from various locations in the study area is presented (Table 1).

Salmonella isolated from different sample types

Higher percentage (45%) of gram negative bacteria suspected to be salmonella were isolated from the intestines in comparison to other organs.

Biochemical characteristics of Salmonella spp

The results of the phenotypic analysis of the isolated gram negative bacteria suspected to be salmonella. Thirty-seven percent (24/65) of the isolates were positive for the biochemical tests performed.

Molecular confirmation of Salmonella isolated from commercial chicken in Jos South

The result of the molecular confirmation of salmonella isolates using specific oligonucleotide primers by PCR is shown in Table 4. Out of the 24 initial suspected salmonella isolates stored on nutrient agar slant, only 6 could be revived for molecular confirmation (four from Kugiya and 2 from Kuru district), of which 2 isolates from Kugiya district (33%) tested positive by PCR.

Table.1 Salmonella isolated from various locations in the study area

Location	No of samples	No of positive samples on XLD
Kugiya	40	23 (58%)
Kuru	40	19 (48%)
Vwang	40	9 (23%)
Gyel	40	14 (35%)
Total	160	65 (41%)

Table.2 Salmonella isolated from different sample types.

	No of sample type positive				
Location	Liver	lungs	spleen	Intestine	**Total**
Kugiya	5	7	6	5	23 (58%)
Kuru	6	5	4	4	19 (48%)
Vwang	2	2	1	4	9 (23%)
Gyel	3	1	5	5	14 (35%)
Total	16 (40%)	15(38%)	16(40%)	18(45%)	65 (41%)

Table.3 Biochemical characteristics of Salmonella spp isolated from chickens in the study area

		Biochemical analysis		
Location	No of isolates	TSI	Urease	No and percentage of positive
Kugiya	23	12	11	11(48%)
Kuru	19	13	10	10(53%)
Vwang	9	5	2	2(22%)
Gyel	14	3	1	1(7%)
Total	**65**			**24(37%)**

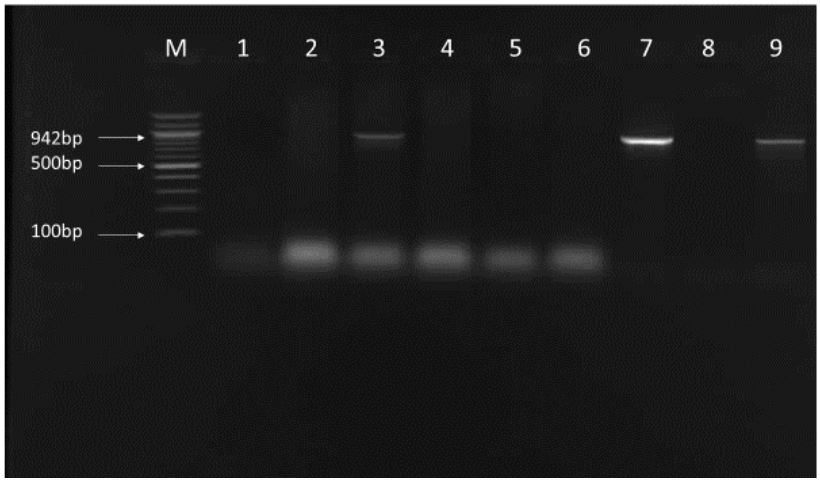
Table.4 Results of the molecular confirmation of salmonella isolates in Jos South LGA using PCR

Location	No isolates	No of PCR positive
Kugiya	4	2
Kuru	2	0
Total	**6**	**2**

Table.5 Antibiotic susceptibility profile of confirmed salmonella isolates in Jos South LGA

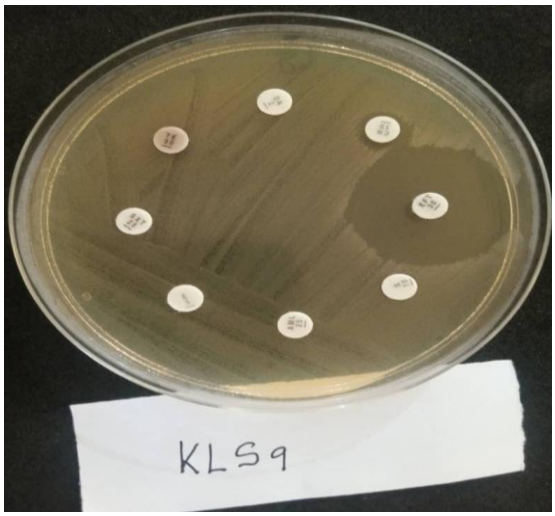
Antimicrobial agents	Concentration µg	No. of strain resistant	No. of strain sensitive
Gentamycin	10	2	0
Sulphamethoxazole/trimethoprim	25	2	0
Tetracycline	30	2	0
Erythromycin	5	2	0
Amoxicillin	25	2	0
Streptomycin	10	2	0
Ceftiofur	30	0	2
Clindamycin	2	2	0

Fig.1 Shows the result of agarose electrophoresis of the positive salmonella amplicons detected by PCR in Kugiya district, Jos South LGA



Confirmation of Salmonella isolates in Jos South LGA using PCR: Positive samples at 942bp. Lanes: M= Maker, 9= positive control, 8= negative control, 3 and 7 = positive samples, 4,5,6 = negative samples

Plate.1 The zone of inhibition observed from the disc sensitivity test is shown in Plate 1.



Antibiotic susceptibility test

The antibiogram of the confirmed salmonella isolates to eight different antibiotics is shown. The confirmed salmonella were found to be resistant to all antibiotics used except ceftiofur® (EFT). The zones of inhibition observed from the disc sensitivity test is shown in Plate 2.

Salmonellosis is a major public health concern and continues to have a serious economic importance in the poultry industry in all countries (Morales and McDowell, 1999). Broilers meat and raw poultry products are considered to be a reservoir of infection to human where *Salmonella* food poising in human is often associated with the consumption of poultry products (Coyle *et al.,* 1988; Olsen *et al.,* 2000). The present study was conducted to investigate the incidence of salmonella species in chicken. All collected samples in the study area were cultured first in XLD. Kugiya district of Jos South LGA had the highest numbers of gram negative bacteria suspected to be Salmonella 58% and 48% respectively. The rate of suspected Salmonellain Kugiya district was (58 %) in this study was comparable to that reported in other studies. Yagoub and Mohamed (1987) examined 1488 samples in the Sudan and isolated 58 *Salmonella* which comprised 3.9% of the total isolates. In another study, Ezdihar(1996) examined 610 samples from poultry in the Sudan and isolated 45 *Salmonella* which counted for 7.4% of the total isolates. The later study showed higher isolation rate compared to the finding of this study and that might be attributed to the large difference in the number of samples collected in both studies.

In the phenotypic analysis of Salmonella isolates, thirty seven percent 24/65 of the isolates were positive to biochemical test. Out of the thirty-four initial isolates stored on nutrient agar slant only six could be revived for molecular analysis where two isolates were confirmed to be Salmonella.

The isolates that were confirmed were further tested for their senility to eight different types of antibiotics. Only one antibiotic Cefttiofur was sensitive to both isolates the remaining seven were 100% resistant.

Two salmonella isolates were detected in Kugiya district of Jos South Local Government Area even though theywere found to be sensitive to the antibiotic ceftiofur® they also showed resistance to the other antibiotics tested (Table).

The present study recommend strict hygienic practices to minimize salmonella infection in poultry farm. Similarly, we advocate that poultry products should be properly washed and cooked before consumption to safeguard human health from infection with salmonella from foods of poultry origin.

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