Molecular Detection of Salmonella Isolated from Poultry Farms in Abia State Southeast Nigeria

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

The study was conducted in six local government areas of Abia State, and this includes Aba north and south, Umunahia south and Ikwuano, Ohafia and Bendel local government areas and three senatorial zones that make up the State, namely, Abia south, Abia central and Abia north respectively. The aim of the study was to detect the presence of Salmonella isolated from poultry farms using molecular method. A total of 1420 samples were collected comprising 800 eggs, 420 cloacal swabs and 200 poultry litter. The samples were processed in the veterinary microbiology laboratory. Universal primer set specific for genus Salmonella 16SrDNA 341F (5'-CCTACGGGAGGCAGCAG-3' and 907R5-CCGTCATTTCTTTRAGTTT-3) was used. The result show that a total of 28 Salmonella were isolated with egg, cloacal swab and litter presenting 18, 7 and 3 Salmonella distribution respectively and isolation rate of 1.97%. There were no significant association (p> 0.05) between rate of isolation and senatorial zone. The molecular study using agarose gel of amplification products shows that there was detection of Salmonella species with bands at 200bp.

KEYWORDS
Salmonella, Poultry Farms, Avian Salmonellosis

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INTRODUCTION

Salmonellae are Gram-negative, short pump rods, non-spore forming, non-capsulated, aerobic and facultative anaerobic organisms and classified under the family enterobactericeae (OIE Manual, 2006). Avian salmonellosiss is the disease of birds caused by members of the genus Salmonella with particular reference to Salmonella pullorum and Salmonella gallinarum (Jordan, 1990). Disease caused by Salmonella infections is most common in Chicks under 2 weeks of age and is rarely seen in birds of 4 weeks of age for pullorum disease while fowl typhoid occurs among adult birds (Khan et al., 1998). The morbidity and mortality vary considerably and deaths are usually less than 20% of the affected group but in exceptional cases can approach 100% (Lulful-Kabir, 2010).

The clinical signs as described by Freitas-Neto et al., (2007) are depression, weakness, anorexia and dropping wings. Others are drop in egg production, prostration and apathy (Ezema et al., 2009). Confirmation
of the diagnosis is by isolation and identification of the causal agent. In chicks dying in the septicemia phase, salmonellae can be isolated directly from the liver, gall bladder or yolk sac, but the intestines and particularly the caecal contents are the most rewarding site to culture (OIE, Manual, 2004).

There are different sources Salmonella can invade farms, Salmonella infection can be from faecal contamination of eggs, introduction of Salmonella into a country via importation of live poultry or hatchable eggs and ineffective vaccination using live Salmonella vaccine (Bensink and Botham, 1983). Rats and mice are documented source of Salmonella and are attracted to the Poultry house by abundance of easily accessible food (Pomery and Nagaraja, 1991). Domestic flies and bettles are both capable of transmitting salmonellae and infection can persist through the insects from one generation to another via eggs and larva (Bustian and Aize, 2007). There has been several molecular methods of characterization of Salmonella with each method having merits and demerit (Sabat et al., 2003). These include; Polymerase Chain Reaction (PCR), Pulsed-Field Gel Electrophoresis (PFGE) and Random Amplification of Polymorphic DNA (RAPD). Polymerase chain reaction (PCR) is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the other template strand. Since DNA polymerase can add a nucleotide only onto a pre-existing 3’OH group, it needs a primer to which it can add the first nucleotide. This requirement makes it possible to delineate a specific region of template sequence that the researcher wants to amplify (NCBI, 2014).

The Pulse-Field Gel Electrophoresis is a standard molecular typing method used to analyze centre to centre transmission and have been successfully used in large scale microbiological and epidemiological investigations (Mac Dougal et al., 2004). The use of Random Amplification of polymorphic DNA (RAPD) is based on the parallel primers that target several unspecified genomic sequences. RAPD has been widely used for the typing of bacterial isolates in cases of disease outbreaks (Lenin et al., 2011). Poultry provides income for small and medium scale farmers in Nigeria, but suffers from major limitations of disease like Salmonella which results in low egg production, low performing breeds and poor feed conversion. This study is necessitated due to the increasing complaint of poultry farmers of incidences of Salmonella in the study area.

Materials and Methods

Study Area

This study was conducted in Abia State Southeast of Nigeria. Abia State lies between latitude 4°40’1 and 6°14’1 north and longitude 7°10’1 and 8°10’1 east. The state is bounded by Rivers State in the south, Ebonyi State in the north, Imo State in the west and Akwa Ibom State in the east. The population of Abia State is 4.3 million, while farming and trading are the major occupation (FRN, 2007).

Study Population

Chickens in poultry farms and hatcheries in Abia State contributed the study population.

Sampling Technique and Sample Collection

A multistage sampling method was employed in this study. In the first stage, Abia State was purposively selected out of
the 5 states in the region based on complaint by many farmers of failures of fowl typhoid vaccine administered to their birds. The sample collection cut across the three senatorial zones that make up the state. In the second stage, two Local Government Areas (Aba south and north, Ikwuano and Umuahia south, Ohafia and Bendel Local Government Areas of Abia State) were randomly selected from each of the three senatorial zones in the state.

Egg, cloacal swabs and litter samples were used for the study. Eggs were collected and placed in polythene and then kept in a plastic box and transported to the veterinary laboratory for processing. Cloacal samples were collected by placing sterile swab stick into the vents of the chicken, gently rotated before withdrawal while the litter was collected by using sterile spatula to collect 10g of litter on the floor of the poultry house into a sterile universal bottle and then transported to the laboratory for processing. The sample collection lasted for a period of 10 months, from July 2014 to April 2015.

Isolation of *Salmonella* from Egg, Cloacal and Litter

Isolation of *Salmonella* was done according to the procedure described by Zancan *et al.* (2000). A loopful of pooled egg were inoculated into peptone water (enrichment broth) and incubated at 37°C for 24 hours. Each cloacal swab sample was inoculated into peptone water (Pre-enrichment broth) and incubated at 37°C for 24 hours. Similarly, for poultry litter approximately 5g of poultry house manure were placed into a universal bottle containing 10ml of physiological buffer saline. The poultry manure samples were inoculated into peptone water and incubated at 37°C for 24 hours. All other procedures apply for eggs, cloacal and litter samples. A loopful of the pre-enrichment broth was inoculated into Rappaport-Vassiliads (RV) broth (enrichment broth) and incubated at 42°C for 24 hours. After incubation, the RV broth was streaked on Macconkey agar and deoxycholate citrate agar. Inoculated plated were incubated overnight at 37°C and observed for colourless (non lactose fermenting) colonies suspicious of *Salmonella*. The *Salmonella* suspected colonies were subcultured to MacConkey media for purification.

**Biochemical Tests**

Biochemical tests were conducted using Simmon citrate agar, Urease agar, Triple sugar Iron, Sugar fermentation test Motility test, Methyl red and Vogues Proskauer test to confirm if the isolates were *Salmonella* phenotypic ally.

**Genomic DNA Extraction Protocol**

Genomic DNA was extracted using the boiling method according to the protocol of Danifor Biotechnology (2012) stock culture of the organism was sub-cultured on MCA and incubated at 37°C for 24 hours. After incubation, three colonies of each isolate were collected and added to 200µl buffer AL (containing lysostaphin) and mixed thoroughly by vortexing.

The suspension was incubated at 56°C for 10 mins, after which 200µl absolute ethanol was added, mixed thoroughly by vortexing and then one milliliter of the mixture was pipette into the DNase mini spin column. The spin column was placed in a 2ml collecting tube and centrifuged at ≥6000Xg for 1min. The flow-through and the collecting tube were discarded. The spin column was placed into a new 2ml collecting tube and 0.5ml buffer AWI, was added and centrifuged for 1 min at ≥6000Xg. The flow through and the collecting tube were discarded again. The
Spin column was placed in a new tube and 0.4ml of buffer AW2 was added and centrifuged at 20,000Xg for 3 mins. The flow-through and the collecting tube were again discarded. The spin column was transferred into a new 1.5 or 2ml micro-centrifuge tube. The DNA was eluted by adding 0.2ml of buffer AE to the center of the spin column and finally centrifuged for 1 min at ≥6000Xg in order to increase the yield.

**Amplification of Target DNA**

The target DNA was amplified by the Polymerase chain reaction (PCR). The procedure described by Promega Cooperation, Madison USA was conducted in a volume of 25µL containing 20µL of genomic DNA from each *Salmonella* isolate. A volume of 30µL of the supernatant was used as template for amplification by PCR assay. The sequence of a pair of primers specific for the genus *Salmonella* (16SrDNA341F5-CCT-ACG-GGA-GGC-AGC-AGC-3 and 907R5-CCG-TCA-ATT-CCT-TTR-AGT-TT-3), Inqaba Biotechnical Industries South Africa was used.

A known *Salmonella* strain was used as positive control. Reactions with the primer were carried out in a total volume of 25μL amplification mixture consisting of 2.5μL of 10X reaction buffer (500mM KCL, 200mM Tris-HCl) of each primer (10mM), 0.6μL of Taq DNA polymerase (fermentase). 3μL of extracted DNA as template and 9.6L of distilled water. Amplification was performed in Techne TC512 thermocycle. The cycling conditions were as follows: 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 90s, elongation at 72°C for 30s, and final extension period for 10min at 72°C. Amplified products were electrophorased in 1.5% agarose gel and a 100-b DNA ladder was issued as a size maker. After staining with ethidium bromide, the gel were visualized and photographed under transilluminator ultra-violet (UV) light with gel documentation apparatus (MB Fermentase USA).

**Results and Discussion**

A total of 1420 samples obtained from 43 farms in Abia State were processed for *Salmonella* isolation. Out of the 1420 samples processed, 40 (2.81%) produce non-lactose fermenting colonies on MacConkey while only 24 (60.0%) were urease negative. The inability of the isolate to hydrolyze urea and the negative indole-reaction obtained is in agreement with Muktaruzzaman *et al*. (2010). The utilization of Simmon Citrate by the isolates was consistent with the finding of Lee *et al*. (2003).

| Table.1 Number of Farms and Local Government Areas Selected For the Study |
|-----------------|-----------------|-----------------|
| **State**       | **Local government area** | **No. Of farms selected** |
| Abia            | Aba north        | 14              |
|                 | Aba south        | 11              |
|                 | Umuahia south    | 6               |
|                 | Ikwuano          | 5               |
|                 | Ohafia           | 3               |
|                 | Bendel           | 4               |
| **Total**       | **6**            | **43**          |
**Table 2** Salmonella distribution location and number sampled from eggs, fecal swab and poultry litter in Abia State.

| Location       | No. sampled | Egg | Cloacal swab | Litter poultry | Isolation rate (%) |
|----------------|-------------|-----|--------------|----------------|--------------------|
| Ohafia         | 60          | -   | -            | -              | 0.00               |
| Bendel         | 60          | -   | 1            | -              | 1.66               |
| Umuahia South  | 180         | 2   | 1            | -              | 1.66               |
| Ikwuano        | 120         | 1   | 0            | -              | 0.83               |
| Aba south      | 610         | 9   | 3            | 2              | 2.30               |
| Aba north      | 390         | 6   | 2            | 1              | 2.30               |
| **Total**      | **1420**    | **18** | **7**        | **3**         | **1.97**           |

**Table 3** Isolation Rate of Salmonella in Poultry Farms in Abia State.

| Senatorial zone | No of samples processed | No positive for *salmonella* isolates | Isolation rate (%) |
|-----------------|-------------------------|---------------------------------------|--------------------|
| Abia North      | 200                     | 2                                     | 1.00               |
| Abia Central    | 420                     | 6                                     | 1.43               |
| Abia South      | 800                     | 20                                    | 2.50               |
| **Total**       | **1420**                | **28**                                 | **1.97**           |

**Fig 1** Map of Abia State showing the three senatorial zones
The 24 isolates agglutinated in *Salmonella* poly O antiserum but did not agglutinate poly H. The distribution of *Salmonella* in the six local government areas ranges from 0.0% to 203%.

The study shows that Aba north and south has the highest with isolation rate of 2.30% each while Ohafia local government area has the least with 0.0% suggesting that farms in Ohafia local government area are *Salmonella* free. This may be due to high management levels practiced by the poultry farmers in that area and right use of vaccine. The isolation rate ranges from 1.00 – 2.50% in the three senatorial zones of Abia State with Abia south having the highest predominant isolation rate of 2.50%. There was no significant association (P > 0.05) between isolation rate and senatorial zone.

From the study, the *Salmonella* isolation rate in Abia State was 1.97% and this is closely related to the findings of Suresh *et al.* (2006) who reported a 1.86% isolation rate in new Delhi, India but in disagreement with Al-Abadi *et al.* (2011), who reported *Salmonella* isolation rate of 9.2% in Dharka, region of Bangladesh. The reason for this low isolation rate may be due to several factors like use of vaccination, good hygiene practices, restriction of poultry farm attendant from moving from one poultry farm to the other and the source of day old chicks.
Universal primer set were used to detect/confirm the *Salmonella* species by Polymerase chain reaction. *Salmonella* species processed by PCR produced bands with amplicon size of 200bp following gel electrophoresis and ethidium bromide staining of the PCR product (Plate 1).

This is in line with the work carried out by Dione *et al.* (2011), but in disagreement with the findings of Zahravi *et al.* (2005). Zahravi *et al.* (2005) in his work carried out in Shiraz City in South of Iran using different primers reported amplification of *Salmonella* DNA with amplification size of 284bp. The difference in amplification size could be due to differences in primer type use. This finding is closely related to that of Kwon *et al.* (2010) who reported the amplification of *Salmonella* with amplicon size of 197bp. The primer type used in this study is the same with that used by Kwon *et al.* (2010).

**References**

Al-Abadi, I.K.M., Al-Mayah, A.A.S. 2011. Isolation and identification of *Salmonella Spp* from Chicken and Chicken environment in Basrah Province. *African J. Biol. Sci.*, 7: 33-34.

Bastian, A., Aize, G. 2007. Animal Microbiology: *Escherichia coli*. Blackwell Scientific Publication Oxford, London, Edinburg, Melbourne Pp: 94-102.

Bensink, J.C., Botham, F.P 1983. Antibiotic Resistant Coliform bacilli, isolated from freshly slaughtered poultry at retail outlets. *Australian Vet. J.*, 60: 80-83.

Danfor Biotechnology. 2012. www.omicsonline.org/biotechnology 2012/

Dione, M.M., Ikumapayi, U., Saha, D., Mohammad, N.L., Adegbola, R.A., Greerts, S., Leven, M., Antonio, M. 2010. Antimicrobial resistance and virulence genes of non-typhoidal *Salmonella* isolates in the Gambia Senegal. *J. Infect. Devel. Countries*, 5: 765-775.

Ezeman, W.S., Onuoha, E., Chah, K.F. 2009. Observations on an outbreak of fowl typhoid in commercial laying birds in Udi, Southeastern Nigeria. *Comparative Clin. Pathol.*, 18: 395 -398.

Freitas Neto, O.C., Penha, R., Filho, C. 2010. Sources of human non-typhoid salmonellosis: A review *Revista Brasilerira de ciencia avicola*, 12: 1-11.

FRN. 2007. www.viewphotos.org/nigeria/flat-map-of-Aba-443.html.

Jordan, F.T.W. 1990. Poultry disease 3rd edition.

Khan, A.H., Bari, M.R., Das, P.M., Ali, M.Y. 1998. Pullock Disease in semi-mature chicken and its experienced pathology. *Bangladesh Vet. J.*, 32: 124-128.

Kwon, Y.K., Kim, A., Kang, M.S., Her, M., Jung, B.Y., Lee, K.M., Jong, W. Kwon, J.H. 2010. Prevalence and Characterization of *Salmonella Gallinarum* in the Chicken in Korea during 2006 to 2008. *Poultry Sci.*, 89: 236-242.

Lee, Y.J., Kim, K.S., Kwon, Y.K., Tak, R.B. 2003. Biochemical characteristics and antimicrobial susceptibility of salmonella gallinarium isolated in Korea. *J. Vet. Sci.*

Lutful-Kabir, S.M. 2010. Avian Colibacillosis and Salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int. J. Environ. Res. Public Health*, 7: 89-114.
MacDougall, L., Fyfe M., Mcintyre, L., Paccagnella, A., Cordner, K., Kerr, A. 2004. Frozen Chicken nuggets and Strips: A Newly Identified risk factor for salmonella Heidelberg infection in British Columbia, Canada. J. Food Protection, 67: 1111-1115.

Mukatuzzaman, M., Haider, M.G., Ahmed A.K.M., Alum, K.J., Rahman, M.M. 2010. Validation and Refinement of Salmonella pullorum (SP) coloured antigen for diagnosis of Salmonella infections in the field. Int. J. Poultry Sci., 9: 801-808.

National Center for Biotechnology information, 2014. Primer based approach for PCR amplification of high GC content gene: mycobacterium gene as a model. Molecular Biology International, http://dx.doi.org/10.1155.2014/937308

OIE. Manual. 2004. OIE Manual Part 2: Pullorum Diseases and Fowl Typhoid. In OIE Manual Diagnostic Tests and Vaccines for Terrestrial Animals. 4th Edition Paris, France.

OIE, Manual. 2006. Fowl typhoid and pullorum disease. In: Terrestrial Manual. Office international des Epizooties (OIE), Paris, France. Pp 538-548.

Pomeroy, B.S. and Nagariaja, K.V. 1991. Fowl Typhoid in: Disease of Poultry, 9th edition. B. W. M. Calnek, H. J. Barnes, C. W. Beard, W. M. Read and H. W. Yoder Jr. eds. Iowa State University Press, Ames, Iowa. Pp 87-99.

Saba, G., Rose, P., Hickey, W.J. 2003. Selective and Sensitive method for PCR amplification of Escherichia coli 16SRNA genes in soil. Appl. Environ. Microbiol., 66: 844-849.

Wong, T.I., Nicol, C., Cook, R., MacDiarmid, S. 2007. Salmonella in uncooked Retail meats in New Zealand. J. Food Protection, 7: 1360-1365.

Zaharvi, S.T., Mahzounleh, M., Saeedzadel, A. 2005. The isolation of antibiotic-resistant Salmonella from intestine and liver of poultry in Shiraz province. where Salmonella serovar typhimurum co-exists in strains with a plasmid of the 23 in-compatibility groups. Microbiol. Pathol., 15: 237-242.

Zancan, F.T., Berchieri, J.A., Fernandes, A. S., Gama, N.M. 2000. Salmonella investigation in transport boxes of day old birds. Brazilian J. Microbiol., 31: 230-232.

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