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Critical segments in the dissemination and transmission of *Salmonella* species from poultry production in Calabar, Nigeria

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Abstract: *Salmonella* species are ubiquitous enteric bacteria, its ability to survive at different stages of the poultry production and food processing chain has been frequently reported in recent years. The present study was undertaken to investigate the points in the segments involved in the production of poultry in Calabar as vital sources of dissemination and transmission *of Salmonella* species to humans. A total of 374 samples were collected from three study segments involved in the production of poultry in Calabar, within the period of August 2013 and May 2014. 170 samples were collected from different points in poultry environment which includes (feed from feeders, water from drinkers, litter/faeces from floor, abattoir reins and drag swab from wall); 136 samples were taken from the live birds (cloacal swab, gut, carcass and egg); and 68 samples were derived from poultry handlers (stool specimens and hand wash). The samples were collected aseptically and analysed for the presence of *Salmonella* species based on the ISO 6579:2002 involving standard bacteriological, biochemical and serologic techniques. The Chi-square, student t-test and simple descriptive statistics were used to analyse the data obtained in this study at 95% confidence level. *Salmonella* species were recovered from 221 (59.1%) of the examined samples involved in the production of poultry in Calabar. There was no significant statistical difference in the rate of recovery of *Salmonella* species from the poultry environmental segment (58.8%), bird segment (55.1%) and poultry handlers segment (67.6%) ($P = 0.230$), implying that these three segments are equally important in the maintenance**,** dissemination and transmission of *Salmonella* species. *Salmonella* species were recovered from all the study sample points implying that all the study poultry sample points are sources of *Salmonella* species. However, the highest recovery rate of *Salmonella* species was observed in the stool sample points of poultry handlers (91.2%) and poultry gut sample points (79.4%) which were significantly different from the rest of the poultry sample points ($P = 0.021$), implying that these two sample points are the major sources of *Salmonella* species and are critical in the dissemination and transmission of *Salmonella* species.

Keywords: *Salmonella* Spp, Poultry, Production Segment, Dissemination/Transmission

1. Introduction

Poultry industry possesses an enormous influence in terms of providing the consumer with good quality protein worldwide. The growth of poultry industry in Nigeria is on a dramatic rise and more than 25% of the total meat production in Nigeria comes from poultry [1]. The poultry industry faces significant challenges in terms of animal health and reduced productivity [2]. The major challenges in poultry production systems worldwide have been frequently attributed to *Salmonella* infection. *Salmonella* organisms have been identified as the major cause of mortality; it is responsible for more than 40% of the death of birds [3] poor chick and egg production [4]

There are other several segments involved in poultry production system and *Salmonella* organisms are not able to establish in all these segments [5].

Other studies have documented the ability of these organisms to survive in animal feeds for more than two years and in the faeces of infected birds for nearly two weeks [6]. *Salmonella* organisms can be maintained in the intestines and gonads of infected birds throughout their life time and the

manifestations may be either overt or asymptomatic and can also transfer the infection to the subsequent generation of birds [1, 6].

These factors therefore render the segments involved in the production of poultry a vulnerable platform for the introduction, establishment and dissemination of the *Salmonella* species. This may result in the infection and re-infection of the birds, as well predisposing the consuming population at risk of developing the disease [7]. Furthermore, the poultry production systems are designed to ensure the massive production of birds usually in confinement. This design is necessary to meet the high protein demand, it also promote the introduction and establishment of the *Salmonella* organisms and hence transmission to the consuming population [8, 9].

Poultry handlers involved in the production of poultry come into direct or indirect contact with the birds or its carcasses. Hence, they also play an important role in the introduction of *Salmonella* to the birds and in the transmission of the infection to the general public. This ranks the *Salmonella* organisms a high priority hazard both in terms of public health and animal health [10].

In spite of the frequent incrimination of poultry as the major reservoir of *Salmonella* species, there is relatively paucity of data with respect to the level of distribution of *Salmonella* species in the various segments involved in the production of poultry. Also, the Hazard Analysis Critical Control Point (HACCP) programs that have been implemented in the western world have proven unsuccessful in the developing countries. In order therefore to implement effective prevention and control strategies, we evaluated the epidemiology of *Salmonella* species to understand the vulnerability of the various segments involved in the production of poultry in the dissemination and transmission of Salmonella infection.

2. Materials and Methods

2.1. Study Area

This study was conducted within the period of August 2013 to May 2014 in Calabar metropolis, Cross River State, Nigeria (Latitude 4.95^{o} North and Longitude 8.32^{o} East) with an elevation of 99 metres above the sea level. It has a population of about 461,796 inhabitants [11]. The commercial poultry production system in Calabar has rapidly developed over the years in terms of size and spread.

2.2. Sample Collection

A total of 374 samples were collected from 34 randomly selected poultry farms within the Calabar, out of the collected samples 170 samples came from 5 different environmental points i.e. (poultry feed from feeders (E1), water from drinkers (E2), litter/faeces from floor (E3), water from carcass reins (E4) and drag swab from wall (E5) 34 samples each) 136 samples were collected from birds segment which included (cloacal swab (S1), gut samples (S2), ready-to distribute samples (S3) and egg samples (S4 34 samples each) in addition to 68 humans samples consisting of stool specimens and poultry handlers hand wash 34 samples each were obtained from 2 poultry handlers segment points.

Samples were collected aseptically in sterile polythene bags and sterile universal bottles were applicable and were kept in a cold box containing ice packs prior to transfer to the laboratory for bacteriological analysis within 12 hours.

2.3. Isolation of Salmonella Species

The isolation and identification of *Salmonella* species from the segments in the commercial production of poultry was performed in according to ISO 6579:2002. The reagents were obtained from HARDY Diagnostics, 1430 West McCoy Lane, Santa Maria, CA 93455, USA.

In the non-selective pre-enrichment stage, a dilution of 1:10 of each sample was made by weighing out 25 g of the solid sample by means of a digital weighing scale (or measuring out 25 ml of the liquid sample by means of a sterile measuring cylinder) with 225 ml of 10% modified Buffered Peptone Water (BPW) into a sterile conical flask and shaken well to mix. This was then allowed in a dark corner over night at ambient temperature.

By means of a sterile pipette, 1 ml and 0.1 ml of the non-selective enrichment was then inoculated into 9 ml of Muller-Kauffmann Tetrathionate-novobiocin (MKTTn) and Modified Semisolid Rapapport-Vassiliadis (MSRV) selective enrichments respectively. These were respectively incubated at 37^{o}C and 42^{o}C in separate incubators for 18 to 24 h.

After incubation, a loop full from each of the selective enrichments was streaked onto both Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate Agar (XLDA) selective plates in order to obtain distinct *Salmonella*-like colonies. On BGA, typical *Salmonella*-like colonies appear as 1-2 mm pink colonies which convert the agar from green to red whereas on XLDA, they appear as pink colonies with or without the presence of black centres (indicating the production of H_2S).

The typical *Salmonella*-like colonies on BGA and XLDA were then picked by means of a sterile wire needle. The needle was used to make a smear on a microscopic slide for gram staining and then used to stab and streak on pre-prepared Triple Sugar Iron Agar (TSIA) and Christensen agar (CA) slants. Those *Salmonella*-like colonies that showed Gram-negative small rods by microscopy, produced alkaline slope/acid butt with or without the production of H_2S and gas, on TSIA slant and urease negative on Christensen agar slant were considered suggestive of *Salmonella*. They were then sub-cultured on nutrient agar slants overlaid with sterile paraffin oil and kept in a cool dark corner prior to further biochemical reactions [12].

2.4. Confirmation of Salmonella Species

Confirmation of *Salmonella* species were carried out based on the standard biochemical techniques in order to identify the isolates which belong to the genus *Salmonella* It involved

the use of Lycine Decarboxilation (LCD) test, β-galactosidase test, Acetone production test and Indole production test.

Serotyping of obtained *Salmonella* isolates was further supplemented by means of the commercially available polyvalent *Salmonella* antisera kit (Denka Seiken Co. Ltd. Tokyo, Japan) specific for all group and type-factor *Salmonella* antigens. A loop full from *Salmonella* isolates that satisfy all the confirmation procedures was then emulsified with one drop of normal saline (0.85% NaCl) on a microscopic glass slide. The preparation was gently stirred and observed for auto-agglutination. If there was no self-agglutination, a drop of the polyvalent antisera was added and gently agitated by rocking back and forth for about three minutes and observed for agglutination. Those that showed agglutination were considered to belong to the genus *Salmonella* [12].

2.5. Statistical Analysis

The data obtained in this study were analysed by means of the Predictive Analytical Software (PASW) 18.0. The simple descriptive statistics was used to analyse the prevalence rate of *Salmonella* species in the samples obtained from the production of poultry. The Chi-square parameter and the student t-test were used to determine the level of significance in the rate of recovery of the *Salmonella* species .*P-values* of less than *0.05* were considered statistically significant.

3. Result

3.1. Prevalence of Salmonella Species

Out of the 374 specimens analysed from the three segments in the production of poultry in Calabar, 221 (59.1%) were positive for *Salmonella* species. The samples from the poultry environmental segment recorded a prevalence rate of 58.8%, the samples from the poultry bird segment, 55.1% and from the poultry personnel segments 67.6%. However, the difference in the rate of recovery of *Salmonella* species among the three poultry segments in the production of poultry in Calabar was not statistically significant ($\chi^2 = 2.939$, $df = 2$, $P\text{-value} = 0.230$).

Among the five poultry environmental segment sample points, the highest rate of recovery of *Salmonella* species occurred in E3 (litter/faeces from floor) (14.1%) and the least from E5 (drag swab from wall) (8.2%) but there was no significant statistical difference in the rate of recovery of *Salmonella* species from the points of the poultry environmental segment ($\chi^2 = 8.986$, $df = 4$, $P\text{-value} = 0.061$). Among the four poultry bird segment sample points, the sample point that revealed the highest rate of recovery came from S2 (gut samples) (19.9%) and the least from S4 (egg samples) (5.9%). The difference in the rate of recovery of *Salmonella* species from the points of the poultry bird segment was statistical significant ($\chi^2 = 24.444$, $df = 3$, $P\text{-value} = 0.000$). Among the two poultry personnel segments, the highest (45.6%) and the least (22.1%) rate of recovery of *Salmonella* species came respectively from P1 (poultry personnel stool samples) and P2 (poultry personnel hand wash samples) respectively. There was also a statistical significant difference in the rate of recovery of *Salmonella* species from the sample points of the poultry personnel segment ($\chi^2 = 17.202$, $df = 1$, $P\text{-value} = 0.000$).

When considering the individual sample points from all the three poultry production segments in Calabar, P1 (poultry personnel stool samples) and S2 (poultry bird gut samples) respectively had the highest rate of recovery of *Salmonella* species of 91.2% and 79.4%, whereas S4 (poultry bird egg samples) had the least rate of recovery of *Salmonella* species of 23.5% (Table 2). However, the difference in the rate of recovery of *Salmonella* species from the individual poultry sample points of the segments involved in the production of poultry was statistically significant ($\chi^2 = 50.482$, $df = 10$, $P\text{-value} = 0.000$).

Table 1. *Recovery of Salmonella species from the various segments in the production of poultry.*

Sources of *Salmonella* samples (n=374)														
Poultry Segments	**Environment (n=170) *P = 0.061***						**Bird (n=136) *P = 0.000***					**poultry handlers(n=68) *P = 0.000***		
Poultry Sample Points	E1	E2	E3	E4	E5	Tot.	S1	S2	S3	S4	Tot.	P1	P2	Tot.
No. Positive	17	22	24	23	14	100	19	27	21	8	75	31	15	46
%. Positive	10.0_a	12.9_a	14.1_a	13.5_a	8.2_a	58.8_b	14.0_c	19.9_d	15.4_e	5.9_f	55.1_b	45.6_g	22.1_h	67.6_b

Subscripts a-h: the same letter subscript indicates no statistical significant difference while different letter subscript indicates significant statistical difference.
a: no statistical significant difference among the environmental sample points (E1 – E5)
b: no statistical significant difference among the three poultry segments
c-f: significant statistical difference among the bird sample points (S1 – S4)
g-h: significant statistical difference among the personnel sample points (P1 and P2)
E1: poultry feed from feeders
E2: water from drinkers
E3: litter/faeces from floor
E4: carcass reins water
E5: drag swab from wall
S2: gut samples
S3: ready-to-distribute samples
S4: egg samples
P1: personnel faecal samples
P2: personnel hand wash samples

Table 2. *Distribution of Salmonella species by sample points in the production of poultry.*

Sample Points	Number collected	Number positive	Percentage positive
E1	34	17	50.0
E2	34	22	64.7
E3	34	24	70.6
E4	34	23	67.6
E5	34	14	41.2
S1	34	19	55.9
S2*	34	27	79.4
S3	34	21	61.8
S4	34	8	23.5
P1†	34	31	91.2
P2	34	15	44.1
TOTAL	374	221	59.1

*† indicate critical poultry sample points (major source of *Salmonella* species) from the segments in the production of poultry in Calabar.
E1: poultry feed from feeders
E2: water from drinkers
E3: litter/faeces from floor
E4: carcass reins water
E5: drag swab from wall
S1: cloacal swab
S2: gut samples
S3: ready-to-distribute samples
S4: egg samples
P1: personnel faecal samples
P2: personnel hand washing

4. Discussion

Salmonella species have been established as a significant problematic zoonosis worldwide and they are responsible for the significant challenges encountered in the food production systems of which the poultry production system is a major concern [12]. In this study, the rate of recovery of *Salmonella* species in the poultry production system within Calabar Metropolis was revealed to be 59.1%. This recovery rate is considerably high and hence suggests that the poultry production systems in Calabar are important reservoirs of *Salmonella* species. This poses a serious threat both to the poultry birds themselves and most importantly to the consumer population. Several studies from different parts of the world have revealed varying recovery rates of the contamination of poultry samples by *Salmonella* species: a prevalence rate of 70.5% was reported in Brazil [13], 63.6% in Ethiopia [14], 53% in Vietnam [15], 35% in Spain [16], 26.6% in Bangladesh [17], 17% in USA [18], 10.1% in Georgia [19], 5% by [20] and 1% in Jamaica [21]. Such varying prevalence rates could be attributed to the differences in the geographical location as well as the standards of hygiene and sanitation practices observed by these regions. However, in 2009 in the Adamawa state of Nigeria, a prevalence rate of 40.8% was reported [3], while this current study carried out within the Calabar metropolis of Nigeria, in 2013-2014 recorded a prevalence rate of 59.1%. This suggests an increasing trend in the rate of recovery of *Salmonella* species from the poultry production system in Nigeria and that the hygiene/sanitation practices are poor. It also suggests that the implementation of control strategies if any is unsuccessful. The rate of recovery of *Salmonella* species from the three segments in the production of poultry in Calabar showed no significant statistical difference ($P = 0.230$), implying that *Salmonella* species are well distributed in the three segments of poultry production in Calabar and hence making them significant sources.

There are several points involved in the production of poultry where the *Salmonella* organisms are capable of colonizing. Once a stage involved in the production of poultry becomes colonized, it puts the poultry at risk of death thereby leading to losses and also renders the poultry unsafe for human consumption. Among the poultry environmental segment sample points (E1, E2, E3, E4 and E5), there was no statistical significant difference ($P = 0.061$) in the rate of recovery of *Salmonella* species. Therefore, the environmental sample points are all good sources of *Salmonella* species.

This current study demonstrated a 50.0% rate of recovery of *Salmonella* species from the poultry feed from feeder (E1) sample points in the production of poultry. Several studies have frequently implicated poultry feeds as an important source of *Salmonella* species [22]. Other researchers demonstrated a rate of 1.4% of *Salmonella* species from poultry feeds [23] and Liljebjelke recorded 3.6% [19] which is not in agreement with the findings in this study. However, the work of Maqsood in Pakistan demonstrated 65.8% [24] and in Zaria, Nigeria researchers recorded 63.8% rate of *Salmonella* contamination of poultry feeds [4] which corroborates the findings recorded in this study. Such high recovery rates are an indication that there is inappropriate or no monitoring of feed in the poultry production chain and that the handling of feed in the poultry production chain is poor. This poses serious threat to the health of the poultry birds and consequence, the consumer population.

In the present study a high rate of recovery of *Salmonella* species (64.7%) from the water from drinker sample points

(E2) were recorded. In Georgia a low recovery rate of 1.8% *Salmonella* species from poultry drinking water [19] was recorded as opposed to the relatively high prevalence rate (55.3%) demonstrated in Nigeria [4]. This suggests that the water reserves in the poultry production systems in Calabar are significantly contaminated probably due to the contamination of tap heads and/or the containers used to fetch water. It also suggests that the poultry drinking water are not appropriately treated or not treated at all and that the hygiene and sanitation standards within the poultry production chain are poor. Inasmuch as the water reserves are exclusively for the poultry birds, such poultry drinking water is an important source of *Salmonella* species leading to the infection of the birds and hence the consumer population.

In USA, a prevalence rate of 29% was reported from poultry litter [25], Liljebjelke *et al.*, recorded 21.8% in Georgia [19] and 2% in Jamaica [21]. However, this study recorded high (70.6%) rate of recovery of *Salmonella* isolates from the poultry litter/faeces sample points (E3) in Calabar. This could be due to the frequent contamination of the floor with infected bird faeces. In spite of the regular changing of litter from floor, the litter or floor in the production of poultry in Calabar are hardly treated. This promotes conditions that favour the survival of the *Salmonella* species. This puts the entire flock at risk of infection which can lead to the massive death of birds [4].

The rate of recovery of *Salmonella* species from the poultry carcass reins sample points (E4) in the production of poultry was recorded to be 67.6% in this study. A study carried out by Bao*et al.,* recorded a rate of recovery of 48% [26] which closely agrees with the result of this study. However, the high rate of recovery of *Salmonella* species from poultry carcass reins may be as a result of the common practice of using an over diluted carcass reins solution and using the same water for a large number of carcass hence reducing its potency of destroying microorganisms. This is very dangerous as entire slaughter line can become contaminated rendering the carcasses unsafe for human consumption.

The recovery rate of *Salmonella* species from poultry house drag swab sample points (E5) were 41.2% our results were in agreement with other studies[19, 27] which reported 39.3% and 40.9% rate of recovery of *Salmonella* species from poultry house drag swabs. Contaminated dust from the walls of the poultry house or any other poultry equipment is an important source of contamination and recontamination of any segment in the poultry production system even after disinfection. This therefore could account for the high prevalence rate of *Salmonella* species from the production of poultry in Calabar.

In this study, cloacal swab sample point (S1) recorded a prevalence rate of 55.9%. This is relatively high compared to the results of other researchers [28, 29, 17] who demonstrated prevalence rates of 4% 19% and 26.3% respectively from poultry cloacal swabs. The relatively high prevalence rate of *Salmonella* from cloacal swabs as recorded by this study suggests that high number the poultry birds in Calabar are infected with the *Salmonella* species.

The recovery rate of *Salmonella* species from the gut sample points (S2) of the poultry bird segment in this study was reported to be 79.4%. This result closely corroborates the 31.6%, 60% and 93.5% respectively [30, 17, 21]. Such high prevalence rates suggest that the birds themselves harbour the *Salmonella* species in their gut to considerable levels and as a consequence can be attributed to the death of birds, reduced egg and chick productivity from the stand point of animal health and also economically. This can also be implicated to the contamination of the poultry environment via faeces and consequently making them significant reservoirs in the transmission of the infection to the other birds in the flock as well as humans and other animals [10].

The rate of recovery of *Salmonella* species from ready to distribute poultry meat sample points (S3) as recorded by this study was 61.8%. Several studies carried out in both the developed and the developing worlds have demonstrated the prevalence rate of chicken carcass contaminated with *Salmonella* to range from 0% to 40% [31]. However, higher prevalence rates of 48.8% and 45.6% have been reported in Karachi and Dubai from poultry slaughter carcass [32, 33]. Nonetheless these high contamination rates are an indication that chicken meat remains an important source of *Salmonella* infection to the consumer population. The result of this study suggests that the poor hygienic and sanitation practices exercised by the poultry abattoirs are important in the contamination of chicken meats destined for human consumption.

In this study, the prevalence rate of *Salmonella* species from the poultry bird segment egg (S4) sample points was 23.5%. This contradicted the works of EFSA, [21, 37] and Suresh [34] who recorded relatively very low prevalence rates of 0.34%, 1% and 1.8% of *Salmonella* contaminated eggs respectively. Nevertheless, the work carried out in Plateau, Nigeria recorded a prevalence rate of 32.5% from retailed eggs [35]. The high rate of *Salmonella* contaminated eggs could be attributed to the high level of environmental contamination as well as the poor hygiene and poor sanitatary practices observed by the egg handlers. Moreover, *Salmonella* Typhimurium and *Salmonella* Enteritidis are the serovars that have been most frequently incriminated to be capable of penetrating egg shells leading to the contamination of the egg content. They are responsible for the contamination of eggs even before they are laid during transovarian transmission [36]. The consumption of such raw or undercooked eggs is a serious hazard to the consumer population.

There was a significant difference in the rate of recovery of *Salmonella* species from the poultry bird segment sample points (S1, S2 S3 and S4) ($P = 0.000$). Therefore, the rate of recovery of *Salmonella* species from the poultry bird gut sample points (S2) was significantly high, thereby implicating it as the major source of *Salmonella* species among the samples of the poultry bird segment.

Poultry handlers have been recognized as the most important epidemiologic factor in the introduction and

dissemination of *Salmonella* species within the poultry production system [38]. In this current study, the rate of recovery of *Salmonella* species from poultry handlers stool and Poultry handlers hand wash sample points was 91.2% and 44.1% respectively. This result reveals that the greater majority of the poultry handlers have a significant level of intestinal carriage of the *Salmonella* species hence making them potential factors in the contamination of all the other poultry segments they come in contact with. The result of this study also extrapolates that the sanitary and hygienic standards of the poultry handlers are very poor. Since the poultry handlers are almost always in contact with all the segments involved in the production of poultry, they play a significant role in the introduction and dissemination of the *Salmonella* organisms in the poultry production system as well as transmission to other humans by account of their contact with other human outside the poultry setting.

Among the poultry handlers segment sample points (P1 and P2), there was a significant difference in the rate of recovery of *Salmonella* species ($P = 0.000$). Since the poultry handlers stool sample points (P1) had the highest rate of recovery of *Salmonella* species, it also serve as a major source of *Salmonella* species among the poultry handlers segment sample points.

The rate of recovery of *Salmonella* species from the individual sample points was significantly different ($P = 0.000$). Therefore, P1 (91.2%) and S2 (79.4%) which had the highest rate of *Salmonella* species from the individual sample points serve as the major sources of *Salmonella* species that are critically important in the dissemination and transmission of the *Salmonella* species to humans and other animals.

5. Conclusion

The results of this study have demonstrated the recovery of significant levels of *Salmonella* species from the poultry segments involved in the production of poultry in Calabar. Hence, all the segments involved in the production of poultry in Calabar are important sources of *Salmonella* to both the birds themselves and the consumer population. However, the birds and the Poultry handlers are critical sources in the dissemination and transmission of *Salmonella* species to humans.

The control of *Salmonella* from all the segments involved in the production of poultry is vital in the management and control of *Salmonella* species within the poultry setting. Hence, prompt identification of *Salmonella* species from the segments involved in the production of poultry is vital in preventing the introduction of the *Salmonella* species in the food chain and hence preventing the transmission of the infection to the consumers.

There is therefore the urgent need for the institution of surveillance *Salmonella* systems for poultry farms and abattoirs in order to regulate the extent of avian and human salmonellosis. In addition, the poultry handlers should be properly educated on the demerits of insanitary and unhygienic habits and the benefits of strict adherence to sanitation and hygiene protocols with the ultimate goal of minimising or eliminating the hazard of *Salmonella* contaminations.

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