Occurrence of *Salmonella* in Raw Chicken Meat from Retail Equipment and Environments in Southern Nigeria Open Markets

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Abstract

*Salmonella* species is one of the most significant food-related pathogens of public health concern, whose leading vehicles of transmission to humans are chicken products. Hence, this study investigated the occurrence of *Salmonella* in chicken meat in correlation to their retailing equipment/environments of open markets located in Warri, Benin City, Akure and Ado-Ekiti metropolis (Southern Nigeria). A total of 680 samples comprising raw chicken carcass (n = 240 muscle tissues), rinsing water (n = 60), hovering houseflies (n = 200) and swabs from retailing table (n = 60), cutting knives (n = 60) and meat storage containers (n = 60) were collected and analysed using standard techniques. *Salmonella* was recovered in 105 samples, presenting a prevalence rate of 15.4% (105/680). Rinsing water (40.0%) had the highest rate, followed by chicken carcass and retailing table (16.7%), storage containers (18.3%), hovering flies (9.0%), and then cutting knives (3.3%). The prevalence of *Salmonella* was highest in Benin City samples (24.7%; P<0.05), followed by Warri samples (15.9%), Ado-Ekiti (11.9%) and Akure (9.4%) being the least. The mean *Salmonella* counts (CFU/mL) per sample revealed that Benin City (0.09 × 10⁵ - 5.49 × 10⁶) yielded the highest *Salmonella* load, followed by Warri (0.00 - 6.11 × 10⁵), Ado-Ekiti (0.00 - 5.49 × 10⁴) and Akure (0.00 - 3.02 × 10⁴). These findings suggest that the occurrence of *Salmonella* in commercial chicken meat is still high in most of the study locations and the rinsing water, tables and storage containers could be potential transmission routes. Adequate thermal treatment measures are recommended before consummation of commercial chicken meat within the regions.

Keywords: enterobacteriaceae; open market; prevalence; poultry; *Salmonella*

Introduction

Globally, poultry meat remains one of the vital sources of essential amino acids, proteins, vitamins and minerals, needed in human diet. As a nutritional balanced food, meats are constantly exposed to microbial contaminations during production or processing (Ogu et al., 2017). Though they serve as important food nutrient for humans, studies have shown that consumption of poorly processed chicken meat and products could serve as a veritable route for most human food-borne illnesses (Stoica et al., 2015). Moreover, subsequent contaminations of the meat usually occur during feather plucking, evisceration, washing, storage environment, and unhygienic processing equipment (Bhaisare et al., 2014; Ogu et al., 2017). The major bacterial pathogens reportedly implicated in samples of poultry meat included *Salmonella enterica*, *Campylobacter*, *Staphylococcus aureus*, *Shigella sp.*, *Escherichia coli*, *Listeria*, *Yersinia enterocolitica*, *Aeromonas* and *Clostridium perfringens* (De Boer et al., 1991; CDC, 2010; Bhaisare et al., 2014; Gonçalves-Tenório et al., 2018). Among these, researchers have identified the genus *Salmonella* as the leading cause of most foodborne illnesses as well as colossal economic losses globally (USDA, 2015). The genus *Salmonella* was first recovered from intestinal samples of infected pigs (classic swine fever) by Theobald Smith in 1855 and colleagues, but was named after the American bacteriologist, D. E. Salmon, a co-worker of Theobald Smith (Arora and Arora, 2014). Although, the
The classification and nomenclature of *Salmonella* is still contentious and developing, currently, the Centers for Disease Control and Prevention (CDC) uses (Popoff et al., 2003; Eng et al., 2015) are adopting the nomenclatural system of *Salmonella* recommended by the World Health Organization (WHO). By this taxonomic system, the two broad species of the genus *Salmonella*, based on the 16S rRNA sequence analysis, are *Salmonella enterica* (type species) and *Salmonella bongori* (Eng et al., 2015). A further study on their genomic relatedness and biochemical properties, regrouped the type species, *S. enterica* into six subspecies; denoted with roman numerals: I, *S. enterica* subsp. enterica; II, *S. enterica* subsp. salamae; IIIa, *S. enterica* subsp. arizonae; IIIb, *S. enterica* subsp. diarizonae; IV, *S. enterica* subsp. bontaeae; and VI, *S. enterica* subsp. indica (Reeves et al., 1989; Hurley et al., 2014; Eng et al., 2015). Among all the subspecies of *Salmonella*, *S. enterica* subsp. enterica (I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Hurley et al., 2014). In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and in cold-blooded animals, and hence are rare in humans (Brenner et al., 2000; Hurley et al., 2014; Eng et al., 2015).

The most clinical feature of salmonellosis is gastroenteritis, which is usually self-limiting, but might be invasive and severe, particularly in children, the elderly and immunocompromised patients (Ogu et al., 2017). The ability of *Salmonella* to cause invasive infection varies with the serovar, the age of patient, and region. Of the over 2,500 known serotypes of *Salmonella* species, *Salmonella enterica* serovars Enteritidis and Typhimurium types (the non-typhoidal *Salmonella* group) remain the most frequently implicated serovars in salmonellosis outbreaks among humans who consume raw or improperly processed animal products such as chicken meat (Panisello et al., 2000; Agada et al., 2014; Eng et al., 2015, Ogu et al., 2017).

Salmonellosis, especially from non-typhoidal *Salmonella* strains, has been traced to numerous sources, but recent reports have narrowed it down to the consumption of poultry and poultry food products (Agada et al., 2014; Akem et al., 2017). Hence, it is pertinent to constantly monitor the whole of such food products. According to previous reports, the increasing level of human salmonellosis stems from the fact that there is a lack of overt signs and symptoms in infected host poultry (Patrick et al., 2004; Dawoud et al., 2011). This scenario is worsened by the obvious absence of well regimented national epidemiological surveillance protocol, thereby beclouding the actual understanding of the spreading routes and carriers sources of non-typhoidal *Salmonella* (Kagambiga et al., 2013; Akem et al., 2017).

Effective management of infection of *Salmonella*, therefore, involves a holistic approach by monitoring the contamination status of commercial raw poultry and associated risk factors in and around open marketing systems, which is prevalent in most developing countries, including Nigeria. Most studies in the past have concentrated on the prevalence of *Salmonella* contamination of poultry farms and production lines (Agbaje et al., 2010; Adesiyun et al., 2014; Agada et al., 2014a; Agada et al., 2014b; Ifeanyichukwu et al., 2016; Akem et al., 2017; Bashir, 2017; Nidaullah et al., 2017), but recent findings revealed that the prevalence thereby was still at relatively low level when compared to the commercial raw poultry carcass exposed to unhygienic retailing materials and environments because of the improved environmental sanitation, hygiene, disease treatment of most commercial poultry farms (Babatunde et al., 2017). Therefore, this study investigated the occurrence of *Salmonella* in commercial raw chicken meat from their retailing equipment and environments within some open markets in Southern Nigeria.

### Materials and Methods

#### Study location

The study locations include four major open markets located in selected major capital cities in and around the Niger Delta region, namely, Effurun main market (Warri metropolis, Delta State), Oja-Oba market, Akure, Ondo State, Oba market (Benin City, Edo State), and Oja-Oba market (Ade-Ekiti, Ekiti State). The study areas are located in the south west (Ondo and Ekiti State) and south south (Delta and Edo States) Nigeria (Fig. 1).

#### Samples size

The method of Bashir, 2017 was adopted. The prevalence rate employed was 20% (Ifeanyichukwu et al., 2016) and the formula for the sample size determination is shown below (Bashir, 2017):

\[
N = PQ / (E/Z)^2
\]

Where \( N \) = Number of sample to be collected, \( P \) = prevalence of previous study 20% (Ifeanyichukwu et al., 2016),

\[
Q = 100 - p = 100 - 20 = 80
\]

\[
E = \text{Allowable error} = 0.05
\]

\[
Z = \text{Standard normal distribution at 95%, CI} = 1.96
\]

From the calculation, using the parameters above, \( N \) was found to be 244. However, the total of samples collected was 680.

#### Sample collection

The samples were collected using the simple random sampling methods from chicken meat and their retailing equipment/environment. The chicken meat included the muscle tissues, while the retailing equipment/environment included the wooden retailing tables, cutting knives, rinsing water, hovering houseflies (*Musca domestica*) and chicken meat storage containers. For each metal and wooden retailing equipment/environment contact surfaces, 10 - 15 cm² area was swabbed thoroughly in accordance with the standard microbiological protocol for examination of food (APHA, 1992). A total of 680 samples, comprising raw chicken carcass (\( n = 240 \) muscle tissues), chicken meat rinsing water source (\( n = 60 \)), hovering house-flies (\( n = 200 \)) and swabs from retailing table (\( n = 60 \)), cutting knives (\( n = 60 \)) and meat storage containers (\( n = 60 \)) were collected.
collected from each market sited within the sampling locations above. The swab heads of the sticks used were rinsed in 10 ml 0.1% buffered peptone water (Faleke et al., 2017). The chicken meat were placed in sterile stomacher bags and sealed appropriately. All the samples were conveyed to the laboratory after collection in black polyethylene bags placed within ice packs to maintain the World Health Organization’s recommended temperature range of 4 °C to 6 °C (WHO, 2010). The sampling was done quarterly between October 2017 to September 2018.

**Bacteriological analysis of samples**

225 ml of sterile 0.1% Buffered peptone water (Becton and Dickinson, USA) was aseptically added to each chicken meat (25 g) contained in stomacher bags (400 ml capacity) mix thoroughly and allow to stand overnight (pre-enrichment) in accordance with the standard protocol (ISO 6579: 2002) for microbiological analysis of food and related products. Thereafter, 1 ml was inoculated into a 9 ml of Rapapport-Vassiliadis Soya broth (RVS Himedia™) and incubated overnight at 37 °C. A loopful from the overnight Rapapport Vassiliadis Soya broth culture was aseptically streaked on previously prepared sterile Xylose lysine deoxycholates Agar (XLD Himedia™) and Salmonella Shigella Agar (SS Himedia™) plates and again incubated aerobically overnight 37 °C. The colonies were examined thereafter for suspected typical characteristics representative of Salmonella colonies on XLD, which appeared coloured (red) or colourless with a black centre (Arora and Arora, 2014; Bashir, 2017). The colonies were enumerated from the replicate plates using colony-counting chamber and the mean counts were expressed as colony-forming units (CFU) according to sample type (mL). The typical colonies were then purified using fresh medium before sub-culturing the pure cultures onto sterile agar slants for further bacteriological characterization.

**Characterization of pure cultures of Salmonella**

The following tests were performed: Gram reaction, Triple Sugar Fermentation (TSI) test, urease test, citrate utilization test, indole production, Methyl Red test, Voges-Proskauer test, according to Bashir (2017).

**Statistical analysis of data**

All the bacterial counts were expressed as colony forming units (CFU)/mL depending on the nature of the sample. Then descriptive statistics was used to express the results as mean and standard deviations of the counts. ANOVA was used to determine the statistical differences of the means counts obtained at level of significance of $p=0.05$.

**Results**

Out of the 680 samples investigated, *Salmonella* was recovered in 105, presenting a prevalence rate of 15.4% (105/680). A breakdown of the total prevalence with respect to sample types showed that rinsing water (40.0%) had the highest rate, followed by chicken carcass and retailing table (16.7%), storage containers (18.3%), hovering flies (9.0%), and then cutting knives (3.3%) (Fig. 2). With respect to sampling location, the prevalence of *Salmonella* was highest in Benin City samples (24.7%) ($P<0.05$), followed by Warri samples (15.9%), Ado-Ekiti (11.9%) and Akure (9.4%) being the least (Fig. 3).

The order of prevalence of *Salmonella* in Warri samples were; rinsing water (46.7%) > chicken carcass (21.7%) > retailing table (13.3%) > hovering flies (8.0%) > storage containers (8.0%) > cutting knives (0.0%) (Table 1). Similarly, Akure rinsing water samples had the highest *Salmonella* prevalence (26.7%), followed by storage containers (20.0%), chicken carcass (8.3%), retailing table (6.7%), hovering flies (4.0%) and cutting knives (0.0%) (Table 2).
In the same vein, a similar trend was observed with the samples from Benin City and Ado-Ekiti (Tables 3 and 4). However, only Benin City samples of cutting knives recorded positive results for *Salmonella* to the tune of 13.7% (Table 3). In all, chicken meat alone accounted for 25% (Benin City, Edo State), 21.7% (Warri, Delta State), 11.7% (Ado-Ekiti, Ekiti State) and 8.3% (Akure, Ondo State) (Tables 1-5).

The bacteriological load of *Salmonella* in the positive samples were enumerated and found to vary significantly with respect to the sampling locations and types. The results *Salmonella* counts in the samples are shown in Tables 5-9. The mean counts (CFU/mL) per samples observed ranged from 0.00 - 6.11 x 10^{5} (Warri), 0.00 - 3.02 x10^{4} (Akure), 0.09 x10^{2} - 5.49 x10^{6} (Benin City) and 0.00 - 5.49 x10^{4} (Ado-Ekiti). It shows that Benin City yielded the highest *Salmonella* load, followed by Warri, Ado-Ekiti and Akure samples. The bacterial counts in the chicken meat were significantly (*p*<0.05) higher in all the samples. Though, the mean counts for the rinsing water samples appears significantly higher in all the samples, they were not statistically different (*p*>0.05) from those of retailing table, hovering flies and storage containers. Zero counts were however recorded for the all the samples locations for cutting knives, except those from Benin City (Table 7).

![Fig. 2. Overall prevalence rate of *Salmonella* in different sample types](image)

![Fig. 3. Overall prevalence of *Salmonella* species in the chicken and retaining/environments (Values with the same letters are not significantly different at *p*=0.05)](image)

| Table 1. Prevalence of *Salmonella* in chicken meat and retailing materials/environment of Effurun market, Warri Delta State |
|---|---|---|
| Locations | Number of samples | Number of positive samples | Prevalence (%) |
| Carcass | 60 | 13 | 21.7 |
| Retailing table | 15 | 2 | 13.3 |
| Rinsing water | 15 | 7 | 46.6 |
| House-Flies | 50 | 4 | 8.0 |
| Cutting knives | 15 | 0 | 0.0 |
| Storage container | 15 | 1 | 6.7 |
| **Overall** | **170** | **27** | **15.9** |

| Table 2. Prevalence of Salmonella in chicken meat and retailing materials/environment of Oja-Oba Market, Akure, Ondo State |
|---|---|---|
| Locations | Number of samples | Number of positive samples | Prevalence (%) |
| Carcass | 60 | 5 | 8.3 |
| Retailing table | 15 | 2 | 13.3 |
| Rinsing water | 15 | 4 | 26.7 |
| House-Flies | 50 | 2 | 4.0 |
| Cutting knives | 15 | 0 | 0.0 |
| Storage container | 15 | 3 | 20.0 |
| **Overall** | **170** | **16** | **9.4** |

| Table 3. Prevalence of Salmonella in chicken meat and retailing materials/environment of Oba market, Benin City, Edo State |
|---|---|---|
| Locations | Number of samples | Number of positive samples | Prevalence (%) |
| Carcass | 60 | 15 | 25.0 |
| Retailing table | 15 | 4 | 26.7 |
| Rinsing water | 15 | 9 | 60.0 |
| House-Flies | 50 | 7 | 14.0 |
| Cutting knives | 15 | 2 | 13.7 |
| Storage container | 15 | 5 | 33.3 |
| **Overall** | **170** | **42** | **24.7** |
Discussion

Salmonella infections still remains a global issue with an annual incidence rate of 93.8 million cases and 155,000 deaths per year (Eng et al., 2015; CDC, 2016; Khan et al., 2018). The extent of the burden of this foodborne disease is globally under-reported, but the challenges are more pronounced in developing countries because of very poor documentation of reported cases of human salmonellosis (Barbour et al., 2015). Humans and animals are constantly exposed to these bacteria via various sources. To date, poultry products, particularly chicken products remain the key source of transmission to humans (Khan et al., 2018). Hence, the needs to continue the investigation of...
commercial chicken meat and associated retailing, with a view augmenting the currently available information within developing countries.

In this study, the occurrence of *Salmonella* species in commercial chicken carcasses, retailing tables, storage containers, knives, rinsing water and hovering flies, were investigated in four States (Delta, Edo, Ondo and Ekiti) within Southern Nigeria. *Salmonella* was isolated from all the analyzed samples. The overall prevalence rate obtained in the four States was 15.4% (105/680). This supports earlier studies on the claims that poorly processed/handled chicken meat is often contaminated by *Salmonella* (Adeyanju and Ishola, 2014; Faleke et al., 2017; Balakrishnan et al., 2018). There were significant differences in the levels of contamination in the various samples with respects to the different locations. From our findings, rinsing water for the chicken meat had the highest level of contamination, while cutting knives were the least. This finding underscores the importance of clean/potable water by retailers for processing of raw chicken carcasses, to reduce the level contaminations by *Salmonella* and other water borne pathogens of public health significance. A very recent study in Lusaka, Zambia, identified the water used for processing chickens as one of the major risk factors of pathogenic bacterial contaminations (Mpundu et al., 2019). Hence, the recovery of *Salmonella* from rinsing water, retailing table, knives and storage containers highlights the unhygienic retailing conditions in which raw chicken carcasses are exposed to at most open markets in the study locations. Our finding on the low occurrence of *Salmonella* in cutting knives contradicts some earlier reports (Nidaullah et al., 2017). Another important finding from our study was the prevalence rate of *Salmonella* in hovering flies at the sampling locations. Previous studies showed the isolation potential of pathogenic microorganisms, including *Salmonella*, from domestic flies and flies are well documented as major vectors for pathogen transmission (Olsen and Hammack, 2000; Ugboagu, et al., 2006; Choo et al., 2011). The occurrence of *Salmonella* in the domestic flies hovering around the retailing tables of the chicken carcasses is therefore of public health concern, too.

Our results also revealed that the contamination of chicken carcass by *Salmonella* varied significantly in each of the sampling location. The highest level of contamination was observed with the samples from Benin City (25%), followed by Warri (21.7%), Ado-Ekiti (11.7%), and Akure (8.3%). This finding is in agreement with earlier reports that contamination of poultry meat varies within and between different countries, globally (Ta et al., 2012; Barbour et al., 2015). The prevalence we obtained were closed to those of 23.1% from Sokoto Main Market and abattoir (Faleke et al., 2017), while those from Warri samples were in line with 11.1% obtained from Calabar Metropolis (Ukut et al., 2010), both in Nigeria. Other prevalence rates reported in Nigeria included 2.0% from Osoogbo market (Adesiji et al., 2011), 6.5% reported from Sokoto Metropolis (Garba et al., 2017), 17% from River State (Omorodion and Odu, 2016). Internationally, the reported prevalence of *Salmonella* in commercial raw chicken meat was 5% in Egypt (Tarabees et al., 2017), 17.41% in Tolima, Colombia (Rodriguez et al., 2015), and 23.7% in Patna, India (Kaushik et al., 2014). Higher occurrences were, however, reported in Tamil Nadu (33.3%) (Balakrishnan et al., 2018), 53.3% in Vietnam (Van et al., 2007) and 41.6% - 54% in China (Yang et al., 2010; Zhu et al., 2014). The differences observed could be attributed to differences in the levels of processors/retailers’ hygiene, environmental sanitations, marketing conditions, sampling methods and protocols of analysis. Similar observations were done by other authors (Adeyanju and Ishola, 2014; Rodriguez et al., 2015).

Furthermore, *Salmonella* counts also varied significantly in relation to the sample types and locations. The highest counts were found in chicken samples, which was significantly different from rest of the samples. This finding is in consonance with the study of Omorodion, and Odu (2016), who reported a total *Salmonella* count of 1.09 x 10⁶ - 4.98 x 10⁶ (CFU/mL). It also corroborates with earlier findings that raw chicken meat are good substrate and vehicle for the transmission of *Salmonella* and other enteric pathogens (Brown, 2000). Additionally, this finding suggests that chicken meat retailed in unhygienic environment, such as open markets, could be a potential reservoir for *Salmonella*. Therefore, the observed level of *Salmonella* was attributed to the lack of proper personal hygienic measures / disinfection of retailing / storage equipment’s coupled with poor environmental sanitations prevalent in most of the open markets within the study locations. Hence, proper handling of raw chicken meat is encouraged to prevent *Salmonella* contaminations.

Conclusions

This study has shown that the occurrence of *Salmonella* in chicken meat retailed in some open markets in Southern Nigeria is still high and varies with the locations. It has also shown that rinsing water and retailing tables/surfaces for chicken meat carry significant risks of contaminating the meat and environment with *Salmonella*. Hence, these findings will provide additional information on the hygienic status of commercial raw chicken carcasses and associated factors within the study locations. This is essential for proactive development of appropriate education for the retailers and the public against *Salmonella* in chicken carcass and its associated transmission routes to prevent possible infections. Moreover, adequate thermal treatment measures are recommended before chicken meat consumption.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

References


