Prevalence and Antimicrobial Susceptibility Profiles of *Salmonella* Species in Poultry Farm Environments in Ghana

Rahman Abilla a*, Adetunde Lawrence Adelani b, Kennedy Gyau Boahen a and Linda Aurelia Ofori a

a Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
b CKT-University of Technology and Applied Sciences, Ghana.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2021/v31i1030347

Editors:
(1). Laleh Naraghi (Ph.D), Iranian Research Institute of Plant Protection, Iran.

Reviewers:
(1). Newton Valério Verbisck, Brazil.
(2). Isabella Goulart Oliveira da Silva, Brazil.

Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here:
https://www.sdiarticle5.com/review-history/82961

Received 20 October 2021
Accepted 23 December 2021
Published 25 December 2021

ABSTRACT

**Background**: Poultry is one of most consumed meat products in Ghana. Outbreaks of *Salmonella* spp infections due to consumption of contaminated undercooked poultry products are of high risk to human health. This study determined the prevalence and antimicrobial resistance patterns of *Salmonella* spp in the poultry environment in the Kwabre East municipality.

**Method**: A total of 114 samples consisting of 38 faecal, 38 dust and 38 feed were taken from a total of 38 farms that consented to the study. Sterile nurse’s caps were worn over the boot to collect faecal and worn over the palm to collect dust samples whilst a sterile spatula was used to collect feed samples. *Salmonella* was isolated using standard culture and biochemical methods. The antimicrobial susceptibility and the minimum inhibitory concentration (MIC) profile was determined using the disk diffusion method under the guidelines and interpretations published by (CLSI, 2018).

**Results**: In all, five (5/38; 13.2 %) of the farms were positive for *Salmonella* with a sample level prevalence of 5.3 % (n=6). Layers were predominantly reared (92.1 %) and all the samples positive for *Salmonella* (n=6; 17.1 %) were from the layers. *Salmonella* strains were prevalent in the dust (n=3; 50 %) followed by faecal matter and then feed. Antimicrobial agents were widely used by
farmers for treatment purposes. *Salmonella* strains were resistant to tetracycline (100 %), trimethoprim-sulphamethoxazole (66.7 %), ampicillin (50 %), chloramphenicol (50 %) and ciprofloxacin (16.7 %). Multi-drug resistance (MDR) was observed among four (n=4; 66.7 %) *Salmonella* strains.

**Conclusion:** The presence of *Salmonella* in poultry environment and the emergence of multiple drug resistant is a major risk for poultry product contamination. Finding from this study will guide decontamination policies in targeting *Salmonella* in the poultry industry. It will be needful to also investigate the molecular mechanism of antimicrobial resistance and characterize the strains using molecular methods.

**Keywords:** Poultry; non-typhoidal *Salmonella*; antimicrobial resistance; prevalence; multi-drug resistant; Ghana.

1. **BACKGROUND**

*Salmonella* is a foodborne pathogen, although ubiquitous, they are normally found in the intestine of animals and is often transmitted through the consumption of contaminated food, especially poultry products that are poorly cooked. *Salmonella* is considered a major cause of food poisoning in Europe [1]. Of concern is the frequent incrimination of *Salmonella* in outbreaks of human salmonellosis [2]. Hence, the presence of *Salmonella* species in the poultry production chain especially at the farm level is of public health concern. The rising prevalence of multi-drug resistance (MDR) serovars in both animals and humans, particularly resistance to clinically important antimicrobial agents, is an emerging concern worldwide [3]. The magnitude and intensity of resistance vary worldwide and are influenced by geographical variation and the rampant use of antimicrobials in both humans and veterinary medicine [4]. More worrying are *Salmonella* strains resistant to antimicrobials, leading to infections in humans that cannot be successfully treated with antimicrobial drugs that they were previously susceptible to [5].

In Ghana, few reports exist on the prevalence and antimicrobial resistance of non-typhoidal *Salmonella* in poultry. Non-typhoidal *Salmonella* are important food-borne pathogens causing gastroenteritis worldwide. *Salmonella* strains that infect poultry are non-typhoidal. Andoh et al. [6], reported 44% Salmonella prevalence in a study conducted in selected poultry farms in Accra and Kumasi, otherwise, most studies have reported non-typhoidal *Salmonella* on humans and meat more than foodborne animals [7].

A systematic literature review of previous studies showed that most of the *Salmonella* strains from poultry products and poultry farms were resistant to several antimicrobials. Since the information on farm level prevalence and antimicrobial susceptibility status can explain the level of public health risk associated with poultry products, this study, therefore, seeks to determine the prevalence and antimicrobial resistance patterns of *Salmonella enterica* in poultry environments in Kwabre East Municipality, Ghana.

2. **MATERIALS AND METHODS**

2.1 Study Design and Study Area

A cross-sectional study was conducted across the communities in the Kwabre East municipality of the Ashanti region from September 2018 to January 2019.

To obtain relevant information from poultry farmers, a purposively structured questionnaire was used. Areas covered included type of farm; knowledge of withdrawal periods, knowledge on antimicrobial resistance, type of poultry kept (broiler or layer), flock size, antimicrobials used for the last one month, type of antimicrobial used, reasons for usage, and frequency of usage.

2.2 Sample Collection

At each poultry farm (n=38), faecal matter was taken using a pair of socks (nurses cap) worn over the boots of farmers, a method that has proven to recover *Salmonella* as compared with taking faecal matter samples directly in farmhouses [8]. At the point of entering into each pen for sampling, the base of the farmer's boots is covered with socks (elasticated nurses round cap, Shanghai Channeled Import and Export CO., Ltd. China) soaked in normal saline (0.90
or (%) kept broilers for meat

°C. An aliquot of the enric

hed BPW

isted using

P

°C for 24 h [11].

2.1%),

ically, and placed in a

P, 10µg), amoxicillin

the proportion of

Salmonella

Salmonella

There were no significant differences between

Salmonella

patients (n=2/38; 5.3%) and feed (n=1/38; 2.6%).

samples collected from dust (n=3/38; 7.9%),

Salmonella

The prevalence of

Bomfa community was high compared to the rest

meat and eggs.

3. RESULTS

3.1 Salmonella Prevalence in Poultry

Farms

The prevalence of Salmonella in this study was

Salmonella

—

fisher’s exact test and p-value < 0.05 was

considered significant [12,13].

3.2 Prevalence of Salmonella in

Environmental Samples

Salmonella was also isolated from environmental

samples collected from dust (n=3/38; 7.9%),

faecal (n=2/38; 5.3%) and feed (n=1/38; 2.6%).

There were no significant differences between

1 to 4000) and largest (≥4001) (Table 1).

There were no significant differences between

the prevalence of Salmonella in broilers (1/3,

33.3%) and layers (12/35, 34.3%) (P = 0.97).

Antibiotic usage was high (35/38; 92.1%) as they

were used for various purposes. Most of the

farmers lack knowledge in withdrawal periods of

purposes (Table 1). When flock size was

stratified, it was found that the prevalence was

not statistically different between the smallest

(≤1000), second smallest (1001-2000), medium

(2001—4000) and largest (≥4001) (Table 1).

With 114 poultry samples analyzed, only 6

samples tested positive for Salmonella with over

all farm level prevalence of 13.2% as shown in in

(Table 2).
Table 1. Prevalence of *Salmonella* stratified by selected factors

<table>
<thead>
<tr>
<th>Selected factors</th>
<th>No of farms</th>
<th>No of <em>Salmonella</em> positive farms</th>
<th>% of farms positive for <em>Salmonella</em></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bird type:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layers</td>
<td>35</td>
<td>5</td>
<td>14.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Broilers</td>
<td>3</td>
<td>1</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Use of antibiotics:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>5</td>
<td>13.9</td>
<td>1.00</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Flock size:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1000</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0.035</td>
</tr>
<tr>
<td>1001-2000</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2001-4000</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>≥4001</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Knowledge of withdrawal period:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>3</td>
<td>33.3</td>
<td>0.123</td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>2</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Complied with meat:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>1</td>
<td>7.7</td>
<td>1.00</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Complied with egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
<td>1.00</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Prevalence of *Salmonella* in poultry farms in Kwabre East Municipality and its surrounding communities

<table>
<thead>
<tr>
<th>Community</th>
<th>No. of farms</th>
<th>No. of sample</th>
<th>No. of positive samples</th>
<th>% of positive samples</th>
<th>% of positive farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bomfa</td>
<td>12</td>
<td>36</td>
<td>3</td>
<td>8.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Ntonso</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nwomase</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nkwanta</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dumanafo</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>11.1</td>
<td>33.3</td>
</tr>
<tr>
<td>Safo</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
<td>50</td>
</tr>
<tr>
<td>Kasem</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mamponteng</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asenua</td>
<td>5</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asonomaso</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
<td>50</td>
</tr>
<tr>
<td>Aboaso</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>38</strong></td>
<td><strong>114</strong></td>
<td><strong>6</strong></td>
<td><strong>5.3</strong></td>
<td><strong>13.2</strong></td>
</tr>
</tbody>
</table>
3.3 Antimicrobial Application on Farms

Most of the poultry farmers in the municipality used antibiotics for various purposes, including prevention and treatment. The commonest antibiotic used by farmers was doxycycline \((n=14; 36.8\%)\) followed by amoxicillin \((n=9; 23\%)\) and enrofloxacin \((n=3; 7.9\%)\) among others. No farm owner had used antimicrobials as feed additives in the catchment area. All farm owners, however, used antimicrobials for therapeutic or prophylactic purposes, especially when one or more birds are sick in the flocks. Interestingly, from the questionnaire administered to the farmers, only two farms had not used antimicrobials for the past three months with no positive sample of *Salmonella*. *Salmonella* was frequently recovered in farms that used only doxycycline (38.5%). None of the farms which use sulphur based drugs tested positive for *Salmonella*.

However, there was no significant differences between farms who used antibiotics and those that did not \((p=1.00)\) as shown in Table 1.

3.4 Antimicrobial Sensitivity Profile of *Salmonella* Isolates

*Salmonella* strains were tested against nine antimicrobial agents commonly used in veterinary medicine according to the questionnaire administered. All strains were resistant \(6/6; 100\%\) to tetracycline, but there were varied resistances to other antimicrobials. The proportion of resistance was higher for trimethoprim-sulfamethoxazole \(4/6; 66.7\%\) than for ampicillin \(3/6; 50\%\), chloramphenicol \(3/6; 50\%\), amoxicillin-clavulanate \(3/6; 50\%\), and ceftazidime \(2/6; 33.3\%\), cefoxitin \(1/6; 16.7\) and ciprofloxacin \(1/6; 16.7\%\) as shown in Table 4. Four \(4\) of the *Salmonella* isolates showed multi-drug resistance (MDR), as they showed resistance to more than three classes of antimicrobial drugs as shown in Table 3. They were resistant to antimicrobials such as chloramphenicol, cefoxitin, ampicillin, trimethoprim-sulphamethoxazole, amoxicillin-clavulanate, tetracycline and gentamicin.

4. DISCUSSION

*Salmonella*’s ability to colonize poultry without displaying any clinical symptoms at the farm level and the resulting contamination of poultry products and the human food chain have been known to be the key causes of human salmonellosis [14,15]. The presence of *Salmonella* in healthy poultry is a key risk factor for potential human salmonellosis outbreaks and epidemiological studies have shown the enormous contribution of infected poultry products to human salmonellosis [1,16].

Fig. 1. *Salmonella* isolated from faecal, dust and feed
Table 3. Multi-antimicrobial resistance pattern of (≥3 classes of antimicrobials) *Salmonella* isolates

<table>
<thead>
<tr>
<th><em>Salmonella</em> isolate</th>
<th>No. of isolate</th>
<th>Resistance patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bo. 2Fa</td>
<td>1</td>
<td>CHL, SXT, AMC, TET</td>
</tr>
<tr>
<td>Bo. 4Du</td>
<td>1</td>
<td>CHL, FOX, AMP, SXT, AMC, TET</td>
</tr>
<tr>
<td>Bo. 4Fe</td>
<td>1</td>
<td>AMP, SXT, TET</td>
</tr>
<tr>
<td>Bo. 30Fe</td>
<td>1</td>
<td>CHL, SXT, AMC, GEN, TET</td>
</tr>
</tbody>
</table>

CHL, Chloramphenicol, SXT, Trimethoprim-sulfamethoxazole, AMP, Ampicillin, AMC, Amoxicillin-clavulanate, FOX, Cefoxitin, GEN, Gentamicin and TET, Tetracycline

Table 4. Antimicrobial resistance pattern of Salmonellae from poultry

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>6(100)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>4(66.7)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3(50)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>3(50)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>3(50)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2(33.3)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1(16.7)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1(16.7)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1(16.7)</td>
</tr>
</tbody>
</table>

In addition, studies show that human salmonellosis can be reduced if adequate control measures involving vaccination, improved biosecurity and surveillance targeting different serovars in poultry are taken [15, 16].

The sample and farm level prevalence of *Salmonella* in this study was 5.3 % and 13.2 % respectively. Previous studies conducted by Andoh et al. [6], reported 25 % and 50.9 % prevalence of Salmonella in Accra and Kumasi respectively. El-sharkawy et al. [17], in a similar study reported 41 % prevalence of Salmonella in Egypt. The exact reason for this difference is hazy; this difference could be due to the choice of farm and the methodology employed. It is also possible that the low prevalence of *Salmonella* in the present study compared with earlier studies could also be due to improved biosecurity measures, regular surveillance and high usage of antimicrobial agents for various reasons. Another noteworthy reason for this low prevalence could be the fact that most of the farms sampled were small-scale farms holding small number of birds unlike large commercial poultry farms where they keep thousands of birds and the feeding and management associated with intensification allows easy dissemination of the *Salmonella* within the farm. Our finding is in concordance with previous report where large farms were significantly linked with high prevalence of *Salmonella* as compared to medium and small-scale farms [18]. Bomfa reported the highest number of *Salmonella* in the municipality. This may be due to the high number of poultry farms examined compared to other communities as well as inadequate biosecurity measures in the community. Cross contamination amongst farms may have also contributed significantly to this rise in prevalence since the farms were close to each other.

The data also show high prevalence of *Salmonella* in layers than in broilers. This may be due to vertical transmission of *Salmonella* during egg laying.

Our study isolated more *Salmonella* from dust as compared with poultry droppings and feed; this affirms the report by Carrique-Mas and Davies [19], who said it is easier to isolate *Salmonella* from dust than from faeces. In previous study, Andoh et al. [6], reported high prevalence in faecal matter as compared with poultry feed and dust. Indeed, there was no significant differences between the frequencies of isolation in the three environmental samples sampled. In contrast, the low prevalence of *Salmonella* in the feed could be due to enhanced biosecurity measures at the feed processing plant. The frequent administration of antimicrobial agents at farm level could be the reason for the low prevalence of *Salmonella* in faecal matter.

*Salmonella* resistance to antimicrobials is a normal evolutionary process, but it is accelerated...
by the selective pressure exerted by the widespread use of antimicrobial drugs, which increased the risk of emergence of antibiotic resistance strains. As a result, a reduction in the effectiveness of several classes of antibiotics for treating infections in humans and livestock is becoming a major problem worldwide [20]. The use of antimicrobials as growth promoters create a selective pressure resulting in bacterial mutation and transference of resistance genes selecting emerging serovars responsible for outbreaks in humans.

High resistance of Salmonella isolates to tetracycline observed in this study could be due to the extensive and indiscriminate use of doxycycline which is in the same class with tetracycline as a growth promoter by farmers. This study contradicts similar works by Alali et al. [21], and Singh et al. [22], which reported 6.9% and 23% resistance to tetracycline, respectively. In contrast, Salmonella isolates showed high sensitivity to less commonly used antibiotics such as ceftazidime, cefoxitin and ciprofloxacin (16.7%). Resistance in 16.7% of the Salmonella strains to ciprofloxacin is concerning due to its importance in human medicine.

Multi-drug resistance (MDR) is defined as antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drug classes [23]. Four isolates (4/6; 66.7 %) were confirmed as multidrug resistant Salmonella per the aforementioned definition. This finding conforms to Schwarz et al. [23], which reported over 70 % MDR Salmonella in Ghana. This finding however, contrasts similar work conducted in Ghana by Wilkins et al. [24], and Saba et al. [7], who found none of the Salmonella isolates to be multi-drug resistance (MDR). ESBL producers show less susceptibility to the quinolones and are usually multi-drug resistant (MDR) [25]. In the present study, three isolates were confirmed by double disk synergy test as phenotypic ESBL producers. These isolates showed resistance to most of the β-lactam drugs used in the study. The genotypic analysis of these isolates proved negative. This finding therefore, correlates with earlier study conducted in Ghana where no ESBL strain was found among Salmonella isolated from poultry [6,26]. However, our finding contradicts earlier study in Bangladesh where ESBL producer strains were in circulation [27]. Our data also contradict earlier study conducted by Mahmood, in Pakistan which found three strains of Salmonella which showed ESBL production by double disk synergy test and were confirmed by genotyping.

5. CONCLUSION

The presence of Salmonella in poultry environment and the emergence of multiple drug resistance is a major risk for poultry product contamination. Findings from this study will guide decontamination policies in targeting reduction of Salmonella in the poultry industry. It will be needful to also to investigate the molecular mechanisms of antimicrobial resistance and characterize the strains using molecular methods.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

The owner of each poultry farm was informed of the study purpose and oral permission was obtained before sampling. Participants consent was documented by responding to the questionnaires.

DATA AVAILABILITY

All data used in the study are available in the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/82961

© 2021 Abilla et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.