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

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Original Research Article

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

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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.

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 Salmonellae 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

%). After moving in a 'figure-of-eight'-like pattern around the pen perimeter, the socks were removed, turned aseptically, and placed in a sterile ziplock bag and labeled. Surfaces of pen, fence and cages were sampled in all flocks using saline moistened sterile nurse caps to gather dust particles and placed them individually in a labelled Ziploc bags.

Using a sterile spatula, approximately 10 g of feed from feeding troughs was gathered at each farm and put into sterile Ziploc bags. All samples were stored in an ice chest containing ice packs to maintain the storage temperature of between 0-4 °C and transported to the Pharmaceutical Microbiology Laboratory of Kwame Nkrumah University of Science and Technology in Kumasi where they were worked on [9].

2.3 Culture and Identification of *Salmonella* Species

Salmonella was isolated and identified using the standard ISO method [10]. All sock (nurse cap) samples, dust samples, and feed samples were put individually in the Ziploc bag, after which 225 ml buffered peptone (BPW) water (CM0509; OXOID Ltd. UK) was added and incubated for 24 h at 37°C. An aliquot of the enriched BPW culture was transferred to selectively modified 10 mL Semi-solid Rappaport Vassiliadis broth (SRV) (ISO, CM1112 OXOID) and incubated for selective enrichment at 41.5°C for 24 h [11]. Each loopful SRV culture was streaked onto Bismuth sulphite agar (modified, CM0201, OXOID) and incubated for 24 h at 37°C. The presumptive *Salmonella* isolates were confirmed with API-20E (bioMèrieux, France) and further serotyped using polyvalent antisera (Poly A-E + Vi, SSI, Denmark).

2.4 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing (AST) profile of each *Salmonella* isolate was determined using locally available antibiotics by the disk diffusion method in Mueller-Hinton agar in accordance with the guidelines and interpretations published by Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2018). The strains were tested for their resistance to the following antimicrobials: ampicillin (AMP, 10 μg), chloramphenicol (CHL, 30 μg), cefoxitin (FOX, 30 μg), gentamicin (GEN, 10 μg), tetracycline (TET, 30 μg), trimethoprim-sulfamethoxazole (SXT, 25 μg), ciprofloxacin (CIP, 10μg), amoxicillin-clavulanate (ANC, 30

μg) and ceftazidime (CAZ, 10 μg). Strains were classified as resistant or susceptible according to the epidemiological cut-off by CLSI.

2.5 Statistical Analysis

Data analyzed from the various activities are provided in the form of summary tables and figures using Microsoft Excel spreadsheets, and GraphPad Prism version 8.0.2., San Diego, CA. Proportions of variables were presentation in percentages. Association of *Salmonella* detection with various factors was tested using Fisher's exact test and *p*-value < 0.05 was considered significant [12,13].

3. RESULTS

3.1 *Salmonella* Prevalence in Poultry Farms

The prevalence of *Salmonella* in this study was 13.2% (5/38) of poultry farms with 5.3% individual sample prevalence as shown in (Table 2). The majority of the farms housed layers grown for egg production (35/38, 92.1%), whereas only (3/38, 7.9%) kept broilers for meat 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). 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 meat and eggs. *Salmonella* isolation in the Bomfa community was high compared to the rest of the communities studied (Table 2).

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).

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 the proportion of *Salmonella* isolated from faecal matter, dust and feed (*p*=0.864) as shown in Fig. 1.

Table 1. Prevalence of *Salmonella* stratified by selected factors

Selected factors	No of farms	No of *Salmonella* positive farms	% of farms positive for *Salmonella*	*P*-value
Bird type:				
Layers	35	5	14.3	0.97
Broilers	3	1	33.3	
Use of antibiotics:				
Yes	36	5	13.9	1.00
No	2	0	0	
Flock size:				
≤1000	17	0	0	0.035
1001-2000	10	2	20	
2001-4000	6	3	50	
≥4001	6	0	0	
Knowledge of withdrawal period:				
Yes	9	3	33.3	0.123
No	29	2	6.9	
Complied with meat:				
Yes	13	1	7.7	1.00
No	25	4	16	
Complied with egg				
Yes	8	1	12.5	1.00
No	30	4	13.3	

Table 2. Prevalence of *Salmonella* in poultry farms in Kwabre East Municipality and its surrounding communities

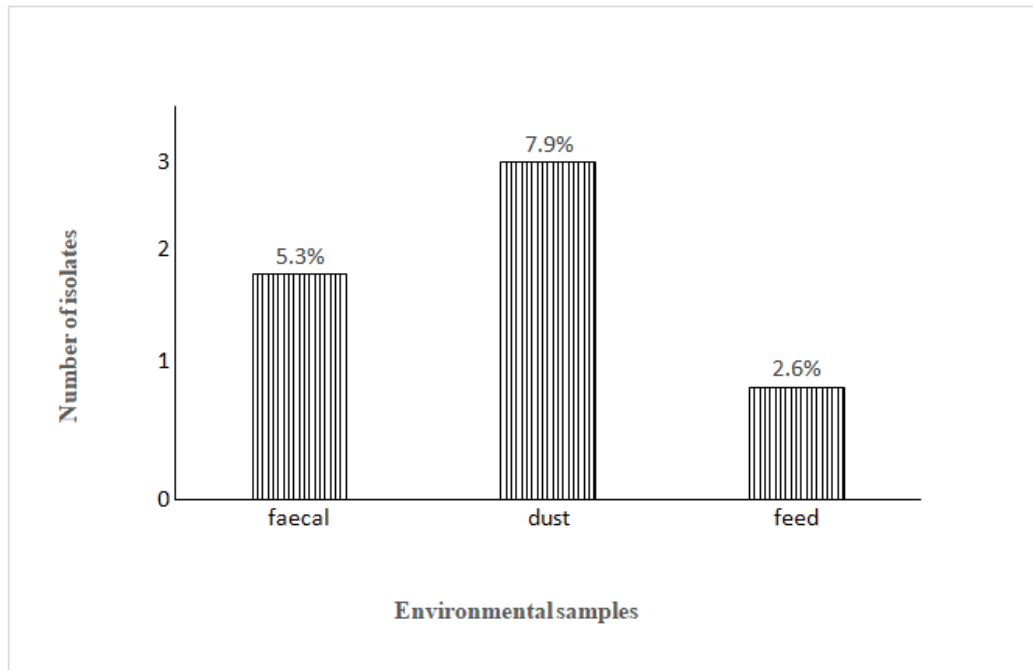| Community	No. of farms	No. of sample	No. of positive samples	% of positive samples	% of positive farms
Bomfa	12	36	3	8.3	16.7
Ntonso	2	6	0	0	0
Nwomase	1	3	0	0	0
Nkwanta	4	12	0	0	0
Dumanafo	3	9	1	11.1	33.3
Safo	2	6	1	16.7	50
Kasem	1	3	0	0	0
Mamponteng	3	9	0	0	0
Asenua	5	15	0	0	0
Asonomaso	2	6	1	16.7	50
Aboaso	3	9	0	0	0
Total	38	114	6	5.3	13.2

Fig. 1. *Salmonella* **isolated from faecal, dust and feed**

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 farmer s 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 Table1.

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 trimethoprime-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].

Table 3. Multi-antimicrobial resistance pattern of (≥3 classes of antimicrobials) *Salmonella* **isolates**

Salmonella isolate	No. of isolate	Resistance patterns
Bo. 2Fa	1	CHL, SXT, AMC, TET
Bo. 4Du	1	CHL, FOX, AMP, SXT, AMC, TET
Bo. 4Fe	1	AMP, SXT, TET
Bo. 30Fe	1	CHL, SXT, AMC, GEN, TET

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

Antibiotics	Resistance patterns
Tetracycline	6(100)
Trimethoprim-sulfamethoxazole	4(66.7)
Ampicillin	3(50)
Amoxicillin-clavulanate	3(50)
Chloramphenicol	3(50)
Gentamicin	2(33.3)
Ceftazidime	1(16.7)
Ciprofloxacin	1(16.7)
Cefoxitin	1(16.7)

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 w here 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.

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