Original Research Article

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *SALMONELLA* SPECIES IN POULTRY FARM ENVIRONMENTS IN GHANA

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 %) and chloramphenicol (50 %) resistance, and Ciprofloxacin resistance (16.7 %). Multidrugresistance (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 and 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 multidrug 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 is *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. 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.

3. Materials and methods

3.1 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.

3.2 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.

3.2 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 of peptone buffered water 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 (ISO, CM1112 OXOID) and incubated for selective enrichment at 41.5 °C for 24 h [11]. Each loopful RV 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 (Biomereux, France) and further serotyped using polyvalent antisera (Poly A-E + Vi, SSI, Denmark).

3.3 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing (AST) profile of each *Salmonella* isolate was determined using 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).

3.4 Statistical analysis

Data analyzed from the various activities are provided in the form of summary tables and figures using Microsoft Excel spreadsheets, and Graph pad prism version 8.0.2 (263). 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.

4. Results

4.1 *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). 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); *Salmonella* isolation in the Bomfa community was high compared to the rest of the communities studied (Table 2).

Table1: Prevalence of *Salmonella* stratified by selected factors

Selected factor	No. of farms	No. of *Salmonella* positive farms	% of farms positive for *Salmonella*	*P*-value
Bird type				
Layers	35	5	14.3	1.00
Broilers	3	0	0	
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 samples(n)	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
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

4.2 Prevalence of *Salmonella* in environmental samples

The number of *Salmonella* isolated was high in 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 figure 1.

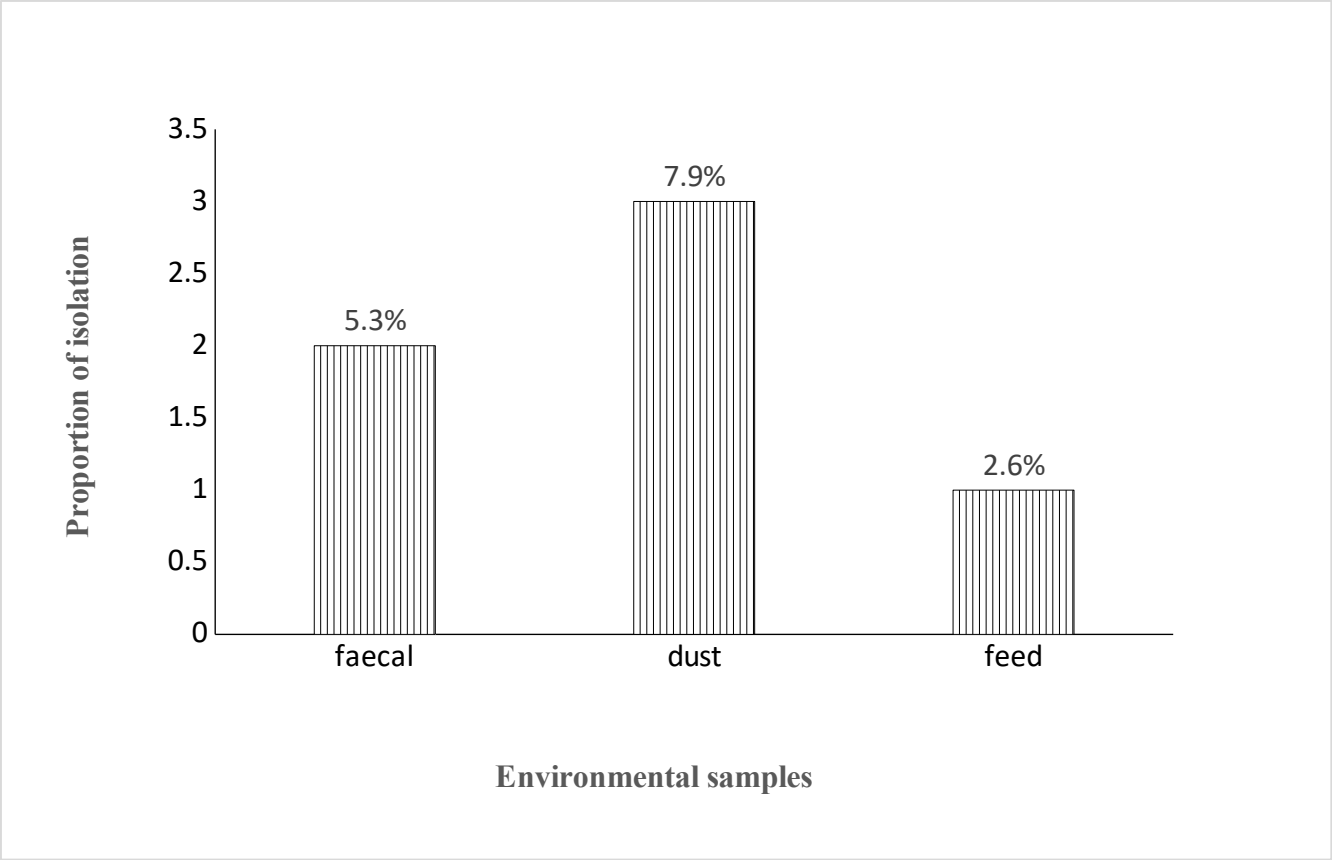

Figure 1: **Salmonellae isolated from faecal, dust and feed**

4.3 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 as shown in Table 3. 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, for the last three months, only two farms had not used antimicrobials with no positive sample of *Salmonella* culture. *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**.

4.4 Antimicrobial sensitivity profile of *Salmonella* isolated

Salmonella strains were tested against nine antimicrobial agents. 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 %), ceftazidime (2/6; 33.3 %), Cefoxitin (1/6; 16.7) and Ciprofloxacin 1/6; 16.7 %) as shown in **Table 3**. Four (4) of the *Salmonella* showed multidrug resistance (MDR), as they showed resistance to more than three classes of antimicrobial drugs. They were resistant to antimicrobials such as chloramphenicol, Cefoxitin, ampicillin, trimethoprim-sulphamethoxazole, amoxicillin-clavulanate, tetracycline and gentamicin.

Table 3: Antimicrobial resistance pattern of Salmonellae from poultry

Antibiotics	Resistant n (%)
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)

5. 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 factorforpotential human salmonellosis outbreaks and epidemiological studies have shown the enormous contribution of infected poultry products to human salmonellosis [1, 16].

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

The sample and farm level prevalence of *Salmonella* in this study was 5.3 % and 13.5 % 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 may be 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. This 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].

The present 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 studies Andoh *et al.* [6], reported high prevalence in faecal matter as compared with poultry feed and dust. There was no significant differences between the frequencies of isolation in the three environmental samples sampled. 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 for microorganisms, but the process 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]. High resistance of *Salmonella* strain 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 in the study area. This study contradicts similar studies by Alali *et al*. [21], and Singh *et al*. [22], which reported 6.9 % and 23 % resistance to tetracycline respectively. In contrast, *Salmonella* strains 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.

Multidrug resistance (MDR) is defined as antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drug classes [23]. Four (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 multidrug resistance (MDR). ESBL producers show less susceptibility to the quinolones and are usually multidrug resistant (MDR) [25]. In the present study, three strains 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, this study contradicts earlier study in Bangladesh where ESBL producer strains were in circulation [27]. The finding also contradicts earlier study conducted by Mahmood [28], in Pakistan which found three strains of *Salmonella* which showed ESBL production by double disk synergy test and were confirmed by genotyping.

6. 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 reduction of salmonella in the poultry industry. It will be needful to also to investigate the molecular mechanism of antimicrobial resistance and characterize the strains using molecular methods.

Data Availability

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

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.