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Research

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Antibiotic usage and Extended-Spectrum Beta-Lactamase-producing *Escherichia coli* in layer birds: a study in the Ashanti Region, Ghana

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Abstract

Introduction: *antibiotic-resistant bacteria are an emerging and growing threat in agriculture, especially in chicken production, due to the overuse and misuse of antibiotics. This research examined the use of antibiotics by chicken producers in Ghana's Ashanti Region and the frequency of Escherichia coli (E. coli) in layer birds that produce Extended-Spectrum Beta-Lactamase (ESBL).* ***Methods:*** *a cross-sectional study was conducted, involving 69 poultry farmers who presented their dead birds for post-mortem examination at the Ashanti Regional Veterinary Laboratory in Amakom-Kumasi. Caecal samples from layer birds were analyzed for ESBL-producing E. coli using microbiological techniques, and data on antibiotic usage and knowledge were collected through questionnaires. Chi-square analysis was performed to assess the association between the prevalence of ESBL-producing E. coli and antibiotic usage.* ***Results:*** *ESBL-producing E. coli was found in 18% of layer bird samples, demonstrating that chickens had antibiotic-resistant bacteria. More of the farmers used multiple antibiotics with tetracyclines being the most often used antibiotic among poultry farmers. Unfortunately, antibiotic usage and antibiotic resistance were little understood by the farmers. There was a significant association (p<0.05) between the presence of ESBL-producing E. coli and multiple antibiotic usage by farmers.* ***Conclusion:*** *this research established a strong association between multiple antibiotic usage and ESBL-producing E. coli in layer chickens which is of serious public health concern. It is recommended that there is a need to advocate for comprehensive antibiotic stewardship in the poultry industry to reduce antibiotic resistance, protect public health and ensure the sustainability of the sector.*

Introduction

The misuse, overuse, under-dosing and indiscriminate use of antibiotics in poultry farming present serious global concerns, with the degree of impact varying across different regions. In Europe, despite stringent regulations, the misuse and overuse of antibiotics persist, contributing to the emergence of antibiotic-resistant bacteria [1,2]. Approximately 30% of all antibiotics used in Europe are administered to livestock, including poultry, with certain countries exhibiting higher usage rates than others [3,4]. Similarly, in Asia, where poultry production is rapidly expanding to meet increasing demand, there is a prevalent overreliance on antibiotics to promote growth and prevent diseases in densely populated farming environments [5]. This excessive use has resulted in the proliferation of antibiotic-resistant strains, posing significant threats to human health through foodborne transmission and environmental contamination [6,7]. In Africa, the challenges are complex and multifaceted, involving issues such as inadequate regulation, weak enforcement, and limited access to veterinary services [8,9]. Underdosing and indiscriminate use of antibiotics are prevalent, driven by factors such as poor awareness, limited resources and weak veterinary infrastructure [10,11]. As a result, antibiotic residues in poultry products and the emergence of antibiotic-resistant pathogens are escalating concerns, heightening public health risks.

E. coli, typically a harmless resident of the digestive tracts of both humans and animals, includes strains that can cause serious illnesses such as diarrhoea, urinary tract infections and life-threatening conditions like sepsis [12,13]. Notably, *E. coli* O157 is a pathogenic strain responsible for numerous foodborne illness outbreaks [14]. Other pathogenic strains include *E. coli* ST131, a significant contributor to urinary tract infections, and *E. coli* K1, which can cause meningitis in newborns [12]. The rise in ESBL-producing bacteria, particularly *E. coli*, is closely linked to the excessive and inappropriate use of antibiotics in both human and animal healthcare [13]. Recent reports showed an increase in the occurrence of ESBL-producing *E. coli* within animal populations, including poultry [15]. Poultry is regarded as a significant reservoir for ESBL *E. coli*, with antibiotic use in poultry farming recognized as a primary factor in the emergence and spread of antibiotic-resistant bacteria [16]. The transmission of ESBL *E. coli* from poultry to humans can occur through direct interaction with contaminated poultry products or via environmental contamination [17]. The consumption of infected poultry products has been identified as a substantial risk factor for human infection with ESBL *E. coli* [18].

The growing prevalence of ESBL *E. coli* among both animal and human populations raises concerns about the effectiveness of existing antibiotic treatments and the potential emergence of extensively resistant strains. In Ghana, the Ashanti Region is notable for its significant poultry farming industry [19], making it a crucial area for investigating the prevalence of ESBL-producing *E. coli* among layer poultry birds. Therefore, this study sought to determine the prevalence of ESBL-producing *E. coli* and antibiotic usage practices among poultry farmers in Ashanti Region.

Methods

Study area

The study was conducted at the Regional Veterinary Laboratory, Kumasi-Amakom in the Ashanti Region which serves as the location for relatively all veterinary-related laboratory analysis including postmortem examination of animals, parasitology, virology and bacteriological analysis of samples. Ashanti Region is one of the sixteen administrative regions of Ghana. It is made up of forty-three districts. Figure 1 shows the administrative map of the districts from which poultry farmers who submitted their dead bird samples to the laboratory for analysis.

Study population

This study was restricted to farmers from Ashanti Region who presented their dead birds to the Regional Veterinary Laboratory situated in Kumasi-Amakom for post-mortem examination. Within the period of this study, samples were received from 31 different district Assemblies out of the 43 districts in the Ashanti Region.

Sampling and sample size

The sampling technique used in this study was the purposive sampling technique which focused on sampling only the dead birds as well as the poultry farmers that participated in providing responses for the questionnaire. In total, 69 poultry farmers brought a total of one hundred and fifty (150) dead birds to the Kumasi Veterinary Laboratory for post-mortem analysis. This number of farmers and the dead birds brought to the laboratory formed the sample size used for this study.

Data collection

Data regarding farmer knowledge and usage of antibiotics was obtained using an existing questionnaire which was the antibiotic resistance: multi-country awareness survey developed by the World Health Organisation [20]. This questionnaire was modified to accommodate its application in this study. The questionnaire was administered to the 69 poultry farmers who brought their dead birds for post-mortem. In the questionnaire, the sections comprised questions on demography of farmers, the knowledge of farmers regarding antibiotics and antibiotic resistance as well as the farmers practices of antibiotic usage. A total of 9 questions were asked about farmers knowledge of antibiotics. The questions had 3 responses each (ie, agree, disagree, don't know). Each question was framed to ensure the correct answer was agreed. For each correct answer agree = 1 mark while each answer of disagree or don't know = 0 mark. Therefore, a total of 9 marks were obtained by farmers that had all answers correct. The categorisation of the farmers knowledge level was done and any farmer with a total score of 0-4 marks was categorised into the "Low knowledge level" group whiles farmers with total marks of 5-9 were categorised into the "High knowledge level" group as described by Chilawa *et al.* [21].

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Regarding sample collection from the birds, 150 paired caecum of in-laying chicken was aseptically clipped and collected during post-mortem. These were transferred into sterile bags, kept on ice in a cooler box and submitted to the bacteriological unit at the same laboratory.

Laboratory analysis

A loopful of caecal content (10 µL) was streaked onto MacConkey agar plates that had 4 µg/mL of cefotaxime (CTX) added to them. The plates were then incubated at 37°C for 18-22 hours to identify potential bacteria that produce ESBL. After that, up to three separate colonies that were digesting lactose were sub-cultured onto nutrient agar plates and incubated in the same way. The purified isolates were then subjected to biochemical tests to confirm *Escherichia coli* (*E. coli*). Discs impregnated with cefpodoxime (CPD) or cefpodoxime mixed with Clavulanic acid (CD) were used for confirmatory testing of ESBL development. ESBL was determined by using the difference between the two inhibitory zone sizes for CPD and CD. An inhibitory zone diameter for CD which was larger than or equal to 5 mm when compared to CPD was deemed as a positive result for ESBL development.

Data analysis

Data obtained from the questionnaire administration and the laboratory analysis were recorded in Microsoft Excel Version 19. Descriptive statistics were carried out on the demographic characteristics. Also, the prevalence of ESBL *E. coli* in layer birds was compared to farmer awareness and antibiotic use employing a Chi-square analysis. Statistical Package for the Social Sciences (SPSS) Version 26 was used for all statistical analysis. All statistical significances were tested at 5% level of significance or 95% confidence interval.

Results

Prevalence of ESBL *E. coli*

Out of the 150 samples analyzed, 39 exhibited pinkish growth on the primary culture (MacConkey plus CTX), indicating a potential presence of ESBL lactose fermenter bacteria. Subsequent testing revealed that 27 out of these 39 samples tested positive for indole, a key confirmation of *E. coli*. Further confirmation through Antibiotic susceptibility testing (AST) confirmed the presence of ESBL *E. coli*, in all 27 samples. Notably, the distinguishing criterion of a CD (Cefpodoxime plus clavulanic acid) - CPD (Cefpodoxime) difference of = 5mm in the zone of inhibition, indicative of ESBL activity, reinforced this confirmation. This comprehensive analysis revealed an overall 18% prevalence of ESBL *E. coli*.

Demographic profile of poultry farmers

The results of the survey revealed a distribution across various demographic and educational categories. In terms of gender, the majority of respondents were male, comprising 88.4% of the sample, while females constituted 11.6%. Regarding age demographics, the largest proportion fell within the 26-35 age range at 31.9%, followed closely by those aged 36-45 at 30.4%. Participants above 55 years old comprised 23.2%, while those below 25 accounted for 8.7%. Educationally, a significant portion had attained Senior High School education (47.8%), followed by tertiary education (33.3%), with basic education and no education representing 15.9% and 2.9% respectively. Regarding training on animal management, 69.6% responded negatively, while 30.4% had undergone such training. Finally, concerning the number of birds managed, most respondents managed between 1,000 and 5,999 birds (60.9%), followed by those with less than 1,000 birds (20.3%), more than 10,000 birds (15.9%) and a smaller proportion managing between 6,000 and 10,000 birds (2.9%) (Table 1).

Awareness of antibiotic resistance among survey participants

Out of the 69 respondents who participated in the survey, a significant majority, comprising 76.8% reported that they had not heard of antibiotic resistance. The remaining 23.2% of the respondents indicated that they were aware of the antibiotic resistance concept.

Poultry farmers' knowledge of antibiotics

Among the respondents, 18.8% expressed uncertainty about whether antibiotics and medicines suitable for people can also be used in animals. Interestingly, 46.4% disagreed with this notion, while 34.8% believed that such antibiotics and medicines are indeed suitable for animals. Only 2.9% of participants refrained from offering an opinion on whether increasing the quantity of antibiotics or medicines would enhance their effectiveness. A majority of 60.9% disagreed with this idea, while 36.2% agreed that greater quantities could improve effectiveness (Table 2). A minority of 5.8% of poultry farmers were uncertain about the statement that antibiotics can stop all diseases. Most farmers comprising 69.6%, disagreed with this notion, while 24.6% believed that antibiotics indeed have the capacity to halt all diseases. Only 2.9% of respondents hesitated to form an opinion on the relationship between the cost of antibiotics/medicines and their quality. A high 59.4% disagreed with the idea that higher costs equate to better quality antibiotics/medicines, while 37.7% agreed that cost reflects quality (Table 2).

Approximately 23.2% of participants were uncertain about whether injections are inherently more powerful than oral antibiotics/medicines. A smaller group, 13.0%, disagreed with this statement, while a substantial 63.8% believed that injections are indeed more potent than their oral counterparts. Among the respondents, 14.5% did not provide an opinion on the prevalence of poor-quality antibiotics/medicines in the market. A striking majority of 82.6% agreed that such products are abundant in the market (Table 2). Approximately 11.6% of participants were uncertain about whether antibiotics can cure Newcastle disease. About 52.2% disagreed with this statement, while 36.2% believed that antibiotics can cure Newcastle disease. A fraction of participants (17.4%) did not express a firm opinion on whether frequent antibiotic use can lead to decreased effectiveness. Although 10.1% of the participants disagreed that excessive antibiotic use can render effectiveness of antibiotics, a substantial 72.5% agreed on this idea. A small group of 5.8% of poultry farmers disagreed with the practice of treating other birds when one is sick to prevent disease, while an overwhelming 94.2% supported this preventive approach (Table 2).

Farmer knowledge level

Based on the responses to the survey questions and an assigned scoring system, the surveyed poultry farmers' knowledge levels were categorized into two main groups. A significant portion, comprising 58.0% of the respondents, fell into the "low level of knowledge" category, indicating a lower level of awareness and understanding regarding antibiotic use in poultry farming. In contrast, 42.0% of the participants belonged to the "high level of knowledge" category, suggesting a more informed and aware segment of the poultry farming community.

Farmer practices towards antibiotic usage

Among the surveyed poultry farmers, antibiotic usage varied by frequency. The smallest percentage of 1.4% administered antibiotics every week, following this, approximately 46.4% reported using antibiotics on a monthly basis, 26.1% used antibiotics every 2-6 months. Additionally, 15.9% used antibiotics exclusively when their birds were sick. Finally, approximately 10.1% relied on veterinary prescriptions for antibiotic use (Figure 2). Regarding the source of antibiotics among poultry farmers, 66.7% of respondents confirmed obtaining antibiotics or prescriptions from a veterinarian or technician, 31.9% indicated they did not receive antibiotics or prescriptions from these professionals and a minimal of 1.4% couldn't recall the source (Table 3).

In our survey, 71.0% of poultry farmers reported receiving advice from a veterinarian, nurse, or pharmacist on how to administer antibiotics to their birds and 29.0% did not seek professional guidance on antibiotic administration during that occasion (Table 3). Poultry farmers provided insights into their practices regarding when they cease treating their birds with antibiotics. The majority constituting 69.6%, indicated that they continue the antibiotic treatment as long as recommended by a veterinarian, emphasizing professional guidance as a crucial determinant in the decision to halt treatment. Additionally, 13.0% follow the manufacturers' instructions for antibiotic cessation, adhering to product guidelines. A smaller but significant 17.4% reported discontinuing antibiotic treatment when they observe signs of recovery or when the bird appears healthy (Table 3).

Poultry farmers employ varied methods for the disposal of unused antibiotics, as indicated in our survey. A notable 34.8% opt to burn these medications, while 15.9% bury them, and 14.5% dispose of them in a garbage bin. Surprisingly, a small percentage of 1.4% choose to keep unused antibiotics. However, a significant 33.3% report the practice of throwing them away (Table 3). In this study, veterinary consultations among poultry farmers, diverse consultation patterns emerged. A substantial 66.7% reported seeking veterinary consultations exclusively when their birds are sick, aligning consultations with therapeutic needs. A smaller proportion, constituting 20.3%, engaged in monthly consultations, emphasizing frequent monitoring and proactive health management. Conversely, a minimal 1.4% never consulted a veterinarian, while another 1.4% described their consultations as regular. A small but noteworthy 4.3% consulted weekly in response to more frequent health challenges and 5.8% mentioned self-treatment when birds didn't respond to initial efforts (Table 3).

Types and class of antibiotics used by farmers

In this survey, poultry farmers revealed their antibiotic usage practices, particularly regarding combinations of antibiotics. The majority, constituting 50.7% of respondents, employed multiple antibiotics simultaneously, reflecting a comprehensive approach to poultry health management. Additionally, 36.2% utilized double combinations of antibiotics, suggesting a strategic approach to addressing specific health challenges. A smaller but notable 13.0% opted for single antibiotics, indicating a simpler approach to antibiotic use (Figure 3). In this study, tetracyclines emerged as the most commonly used class, with 76.8% of respondents utilizing them. Aminoglycosides were closely followed, employed by 73.9% of farmers, while penicillins were used by 62.3%. Macrolides represented another significant class, with 50.7% of participants reporting their use. Fluoroquinolones and polypeptide antibiotics were also employed, though by fewer farmers, at 23.2% and 17.4%, respectively. Phenicol derivatives were the least commonly used, reported by only 2.9% of respondents (Figure 4).

Association between ESBL status and combinations of antibiotics

The chi-square analysis revealed a statistically significant (χ^2= 6.038, p = 0.049) association between ESBL status of farms and the combination of antibiotics used by farmers. In this vein, for the poultry farmers using double combinations of antibiotics, 27.5% were classified as ESBL-negative, while 8.7% were ESBL-positive. For those employing multiple antibiotics, 30.4% were ESBL-negative and 20.3% were ESBL-positive. In contrast, poultry farmers using single antibiotics were all classified as ESBL-negative, representing 13.0% of this subgroup (Table 4).

Discussion

This current study focused on farmers antibiotic usage and prevalence of ESBL-producing *E. coli* in layer birds in the Ashanti Region of Ghana. The 18% prevalence of ESBL-producing *E. coli* in layers in the Ashanti Region, Ghana adds to the existing data on ESBL *E. coli* and highlights the threat posed by this bacterium to animals, humans and the environment. The prevalence obtained is significantly different from a previous study in 2009 in Accra, Ghana which did not detect any ESBL-producing *E. coli* from 103 samples [22]. However, a higher prevalence of 29% was reported in a study conducted in Asante Akyem Agogo in 2015 among broilers [23]. Similarly, a 38.5% detection rate of ESBL *E. coli* has been reported in layers from small-scale poultry farms in Maiduguri, Nigeria [24] while in India a significantly higher prevalence of 42% ESBL *E. coli* has been reported in layer chickens [25]. Additionally, prior research in Bangladesh showed even higher prevalences in commercial layer hens, 64% in the Bogura District, 71% in the Gazipur District, and 65% in the Joypurhat area [26]. Furthermore, a study in Tunisia reported a 35% prevalence of cefotaxime-resistant *E. coli* isolates, which were identified as ESBLs and plasmidic AmpC beta-lactamase (pAmpC-BL)-producing strains [27]. Additionally, the prevalence of ESBL-producing *E. coli* in poultry workers and chickens in Abuja, Nigeria, was 37.8%, followed by the environment at 24.3% [28].

In contrast, some studies have reported lower prevalences. For instance, a study in Bogor reported an 8.6% prevalence of ESBL-producing *E. coli* in slaughterhouses [29] and another study in Bogor reported a 6% prevalence in broiler chicken faeces [30]. Furthermore, a study in Dar es Salaam, Tanzania, found that out of 212 identified *E. coli*, 4.7% were confirmed to be ESBL-producing [31]. As low as 1.47% of ESBL-producing bacteria in chickens was observed in Marseille, France [32]. These discrepancies include variations in sample sizes and antibiotic use across chicken farms in the various research regions. The results of this study suggest that antibiotic prophylaxis is often used for extended periods on farms, which might lead to drug-resistant bacterial pathogens in the layer birds. Since farm hygiene is so important in minimizing pathogen contamination, the biosecurity measures and worker hygiene associated with the high incidence of ESBL-*E. coli* in this investigation might be explained by these factors. The findings of this research suggest that *E. coli* may be used as a model organism to track the transmission of genes that cause antibiotic resistance. These bacteria and the genes that confer resistance may find their way into people's bodies via the food chain, posing a serious threat to public health. Additionally, the use of bird droppings as organic fertilizer for vegetable gardens is another potential route of environmental contamination [33]. This underscores the importance of stringent biosecurity measures and judicious use of antibiotics in poultry farming to mitigate the spread of antibiotic-resistant bacteria.

The findings of this study indicated that over half of the farmers demonstrated limited knowledge about antibiotic usage, whereas only a small portion had a high level of understanding. This finding is consistent with Chilawa *et al.* [21] who also found that many poultry farmers in Kitwe, Zambia, had insufficient knowledge of the proper use of antibiotics. Nevertheless, their findings indicated a marginally smaller proportion (46.2%) of farmers exhibiting limited knowledge compared to the results of our study. The disparity in the results may be attributed to the divergence in the attributes of the farmers in the two locations. The farmers' limited understanding may be attributed to their educational backgrounds, as the majority of them possessed only a secondary school education. This study also demonstrated that nearly all of the farmers were acquainted with antibiotics, however, a minority knew about antibiotic resistance. This finding agrees with previous studies that reported low awareness of Antimicrobial Resistance (AMR) among poultry farmers in Zambia, Malaysia and other countries [21,34-37]. This low level of knowledge about AMR may be due to limited education and awareness, as many poultry farmers in this study lacked a higher level of education (tertiary level), where less than a third achieved it. A study of Hassan *et al.* [38] confirmed that the education level of the poultry farmers was significantly associated with their knowledge and practice of AMU and AMR. The limited knowledge about AMR within the poultry farming community highlights the need for improved educational initiatives and the implementation of antibiotic stewardship efforts.

The most frequently used class of antibiotics in this study were tetracyclines, specifically oxytetracycline, tetracycline, and doxycycline, followed by penicillins and aminoglycosides. This is in line with previous studies which found the most used antibiotic in Zambia being tetracycline (86.4%) [21]. Similarly, a study that was conducted in Ethiopia reported tetracycline as one of the most commonly used antibiotics by poultry farmers [39]. In Nigeria, a study found that tetracycline was the most commonly used antibiotic by animal farmers in their livestock including chickens followed by ciprofloxacin, ampicillin and gentamycin [40]. Tetracyclines are a cost-effective option for large-scale poultry farming operations due to their affordability compared to other antibiotics. Additionally, tetracyclines are readily accessible in various forms, such as oral formulations and injectables, making them convenient to administer to poultry. The widespread usage of tetracyclines among chicken farmers is likely due to this reason. A study conducted in Iran found that chicken producers commonly use tetracyclines as antibiotics. The main reasons for its use are the easy availability and affordability of these medications [41]. Findings Sangeda *et al.* and Granados-Chinchilla *et al.* suggest that the excessive use of tetracyclines in the veterinary sector contributes to an elevated risk of AMR [42,43]. Additional reports have highlighted that the overutilization of tetracyclines in poultry farming has played a role in the development of microbial resistance to these antibiotic agents [44-46].

Farmers who used multiple antibiotics showed the highest occurrence of ESBL-*E. coli* in the study, followed by those who used two antibiotics. This suggests that the widespread use of multiple antibiotics in poultry farming either as a preventive measure or as a form of treatment by farmers, is not a solution for reducing the formation of ESBL-*E. coli*. This study suggests that the incorrect use of a mixture of several types of antibiotics may have a major impact on the spread of ESBLs within bacterial populations [46].

Insights into antibiotic usage, resistance, and the prevalence of ESBL-producing *E. coli* among chicken producers in the Ashanti Region of Ghana were revealed through this study's findings. Nevertheless, due to the utilization of a comparatively smaller sample size and the method of sample collection, the results may not accurately reflect the entirety of the Ashanti Region and the country as a whole. In addition, cross-sectional studies are unable to forecast the results of a survey or any potential treatments. Furthermore, this study was limited by the unavailability of resources to molecularly ascertain the genetic disposition of the ESBL *E. coli* identified in this study which would have been a substantial addition to the narrative of the occurrence of this organism in poultry birds in the region.

Conclusion

This study reports a significant prevalence of antibiotic usage in poultry among farmers residing in the Ashanti Region, with tetracyclines emerging as the most commonly administered medication. As a result, the majority of poultry farmers had limited understanding regarding the use of antibiotics and the development of antibiotic resistance. This resulted in the presence of ESBL-producing *E. coli* in 18% of the farms. It is therefore necessary to establish antibiotic stewardship programmes and antibiotic surveillance systems, as well as educate farmers to address this rapidly increasing issue to guarantee the safety of food and protect public health.

What is known about this topic

- *The overuse and misuse of antibiotics in poultry farming are recognized as significant contributors to the emergence of antibiotic-resistant bacteria;*
- *Antibiotic-resistant strains, such as Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli, are prevalent in poultry worldwide, with the excessive use of antibiotics in various regions leading to serious health risks through foodborne transmission;*
- *Many poultry farmers lack adequate understanding of antibiotic resistance and its implications, often using multiple antibiotics without proper guidance, which exacerbates the problem of antibiotic resistance in the poultry industry.*

What this study adds

- *Occurrence of Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli in layer birds among poultry farmers in Ghana's Ashanti Region, highlighting a significant public health concern related to antibiotic resistance in local poultry production;*
- *Farmers frequently use multiple antibiotics, with tetracyclines being the most commonly used, and demonstrates a significant association between the prevalence of ESBL-producing E. coliand the practice of using multiple antibiotics;*
- *The research underscores a critical lack of understanding among farmers regarding antibiotic resistance, indicating the urgent need for educational interventions to promote better antibiotic stewardship in the poultry industry.*

Competing interests

The authors declare no competing interests.

Authors' contributions

All authors have read and agreed to the final manuscript.

Tables and figures

Table 1: demographic characteristics of farmers

Table 2: farmers' response to queries on antibiotics

Table 3: farmers response to practices regarding antibiotic usage

Table 4: association between ESBL status and combinations of antibiotics used

Figure 1: map of districts from which farmers and bird samples originated

Figure 2: frequency of use of antibiotics

Figure 3: farmers' response type of antibiotic combinations used

Figure 4: class of antibiotics frequently used by farmers

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Table 1: demographic characteristics of farmers

Variable	**Categories**	**Frequency (n)**	**Percentage (%)**
Gender	Female	8	11.6
	Male	61	88.4
Age	Below 25	6	8.7
	26 - 35	22	31.9
	36 - 45	21	30.4
	46 - 55	4	5.7
	Above 55	16	23.2
Educational level	No education	2	2.9
	Basic education	11	15.9
	Senior high school	33	47.8
	Tertiary education	23	33.3
Training in animal management	Yes	21	30.4
	No	48	69.6
Number of birds	Less than 1,000	14	20.3
	1,000 - 5,999	42	60.9
	6,000 - 10,000	2	2.9
	More than 10,000	11	15.9

Table 2: farmers' response to queries on antibiotics			
Statements	**Response (n, %)**		
	Agree	**Disagree**	**Don't know**
If one bird is sick, then others should be treated also to prevent disease	65 (94.2%)	0 (0.0%)	4 (5.8%)
Using antibiotic too often, it may stop being effective	50 (72.5%)	7 (10.1%)	12 (17.4%)
Antibiotics can cure Newcastle disease	25 (36.2%)	36 (52.2%)	8 (11.6%)
There are many poor-quality antibiotic/medicines in the market	57 (82.6%)	2 (2.9%)	10 (14.5%)
Injections are always more powerful than oral antibiotics/medicine	44 (63.8%)	9 (13%)	16 (23.2%)
The more an antibiotic cost, the better it is	26 (37.7%)	41 (59.4%)	2 (2.9%)
Antibiotics stops all diseases	17 (24.6%)	48 (69.6%)	4 (5.8%)
Increasing the amount/ quantity of antibiotic/medicine will make it more effective	25 (36.2%)	42 (60.9%)	2 (2.9%)
All antibiotic/ medicine suitable for people can be used in animals	24 (34.8%)	32 (46.4%)	13 (18.8%)

Table 3: farmers response to practices regarding antibiotic usage			
Practices	**Response**	**Frequency**	**Percentage (%)**
Obtain prescriptions for antibiotic usage	Yes	46	66.7
	No	22	31.9
	Can't remember	1	1.4
Get advice from vet or vet nurse on how to administer antibiotics	Yes	49	71
	No	20	29
Frequency of veterinary consultation in antibiotics usage	Monthly	14	20.3
	Never	1	1.4
	Regular	1	1.4
	Weekly	3	4.3
	When birds are sick	46	66.7
	When I treat myself and birds are not getting better	4	5.5
When do you have to stop antibiotic administration	Continue as long as veterinarian says	48	69.6
	Follow manufacturer's instructions	9	13
	When I see bird recovering or looking healthy	12	17.4
Mode of disposal of unused antibiotics	Burn	24	34.8
	Bury	11	15.9
	Garbage bin	10	14.5
	Keep it	1	1.4
	Throw away outside	23	33.3

Table 4: association between ESBL status and combinations of antibiotics used					
Type of Antibiotic	**ESBL Status**		**Total**	**p-value**	**Chi-Square value**
	Negative	**Positive**			
Double	19 (27.5%)	6 (8.8%)	25 (36.3%)	0.049	6.038
Multiple	21 (30.4%)	14 (20.3%)	35 (50.7%)		
Single	9 (13.0%)	0 (0.0%)	9 (13.0%)		

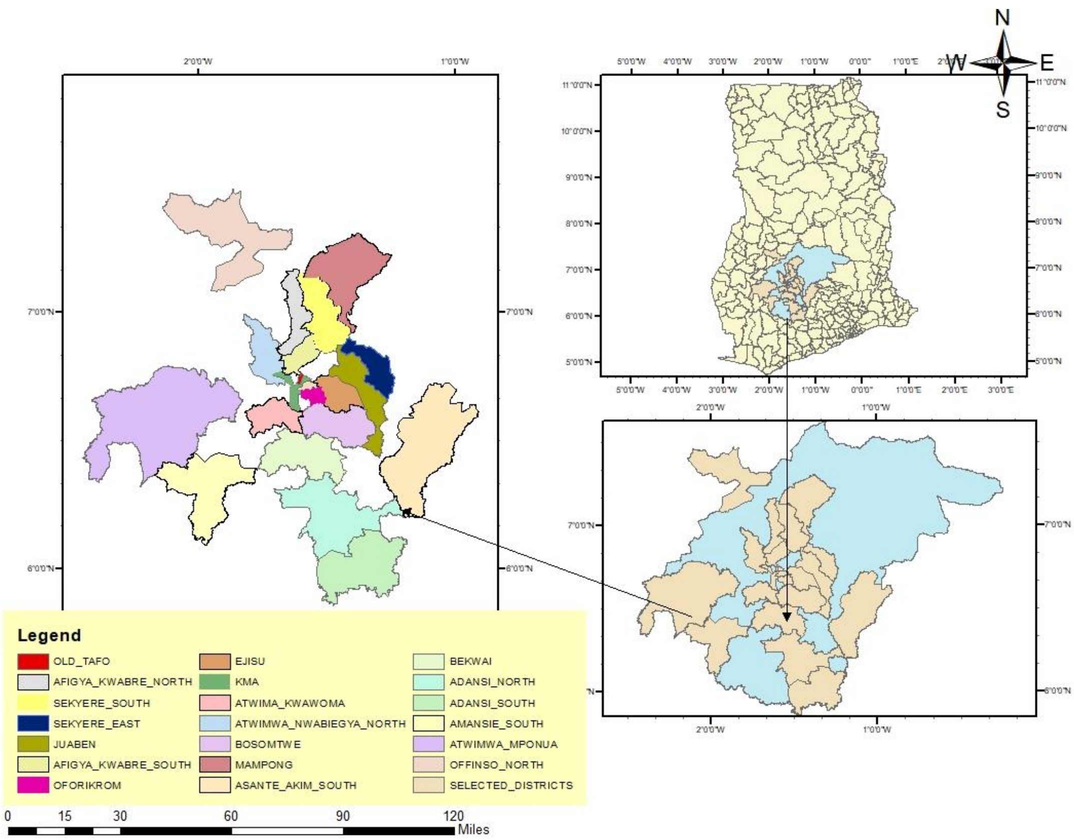

Figure 1: map of districts from which farmers and bird samples originated

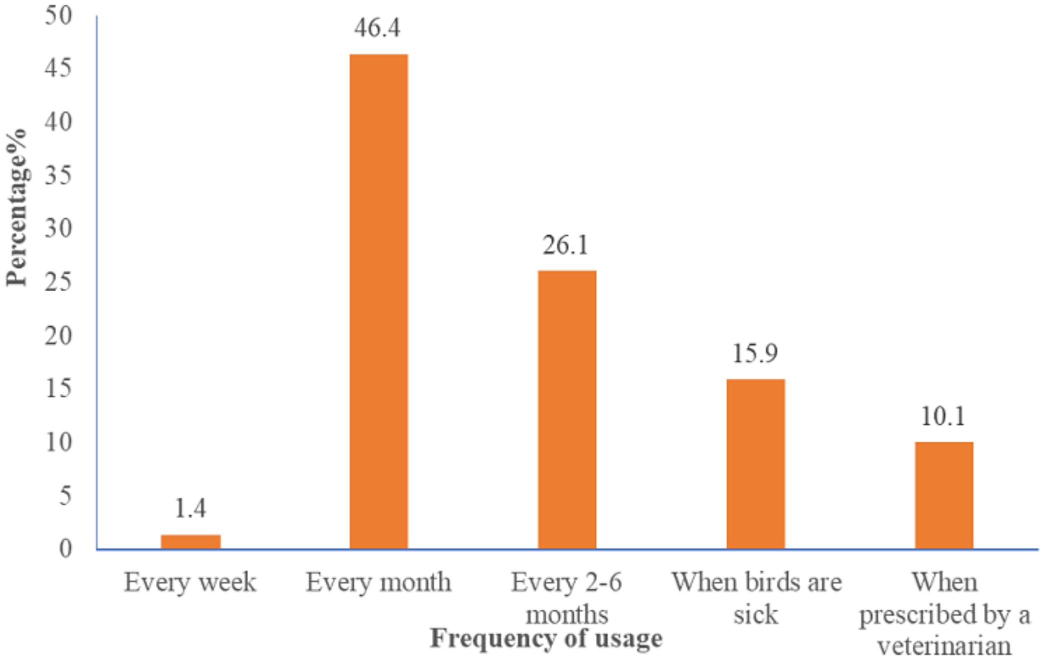

Figure 2: frequency of use of antibiotics

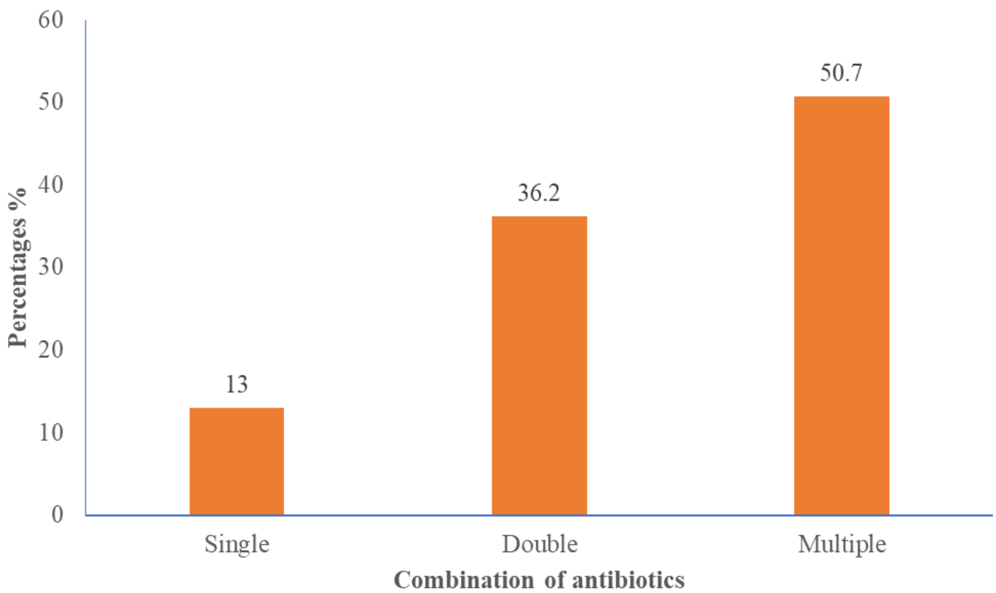

Figure 3: farmers' response type of antibiotic combinations used

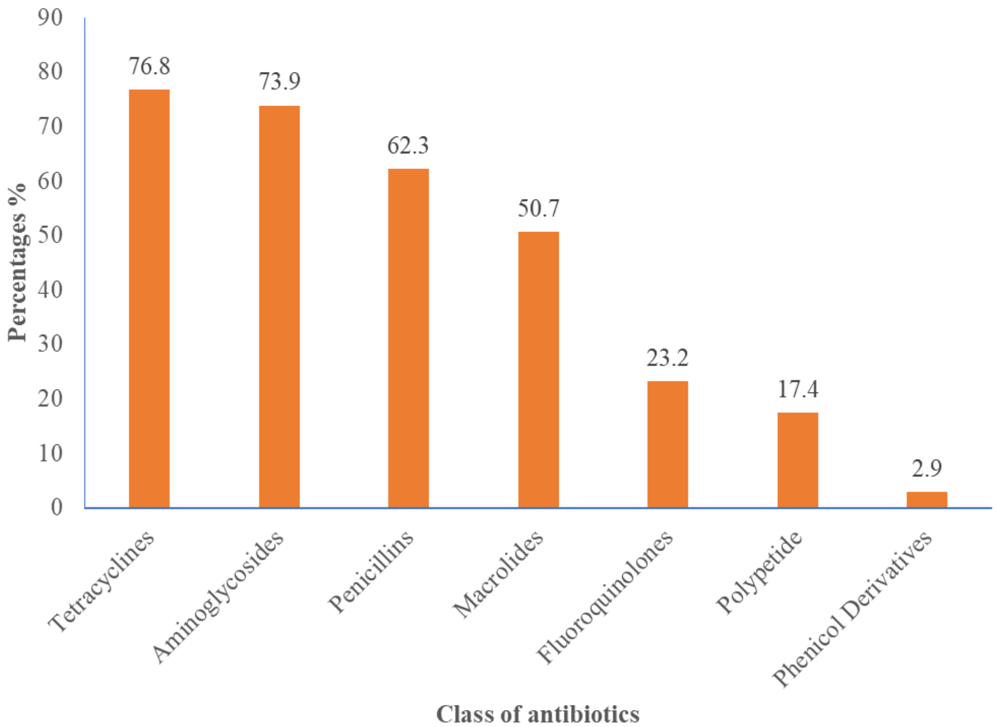

Figure 4: class of antibiotics frequently used by farmers