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Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt

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Abstract In Egypt, poultry production constitutes one of the main sources of pollution with veterinary antibiotics (VAs) into the environment. About 80 % of meat production in Egypt is of poultry origin, and the potential environmental risks associated with the use of VAs in these farms have not yet been properly evaluated. Thus, the main purpose of this research was to evaluate the prevalence of antibiotic-resistant enteric key bacteria and the incidence of residual antibiotics in poultry farm environmental samples and to determine whether fertilizing soils with poultry litter from farms potentially brings ecological risks. From December 2011 to September 2012, a total of 225 litter, bird dropping, and water samples were collected from 75 randomly selected boiler poultry farms. A high prevalence of *Escherichia coli* (n=179; 79.5 %) in contrast to the low prevalence of *Salmonella* spp. (n=7; 3.1 %) was detected. Amongst *E. coli* isolates, serotypes O142:K86, O125:K70, O91:K, and O119:K69 were the most common. Meanwhile, *Salmonella enterica* serotypes emek and enteritidis were recovered. The antibiograms using the disc diffusion method revealed significantly more common resistant and multi-resistant isolates in broiler poultry farms. Residues of tetracycline and ciprofloxacin were detected at 2.125 and 1.401 mg kg^{-1} mean levels, respectively, in environmental samples contaminated with *E. coli*-resistant strains by HPLC. The risk evaluations highlighted that tetracycline residues in poultry litter significantly display environmental risks with a hazard quotient value above 1 (1.64). Our study implies that ineffective implementation of veterinary laws which guide and guard against incorrect VA usage may potentially bring health and environmental risks.

Keywords Poultry farms · Veterinary antibiotics · Enteric bacteria · Drug resistance · Residue · Environmental risk evaluations

Introduction

Poultry industry has steadily expanded in Egypt as it has been encouraged by local governments to ensure a stable supply of animal products and farmers' income. By 2006, it was estimated that 84 % of the total meat production was of broiler chicken origin (Maged and Hamdey 2006). Unfortunately, the expanding poultry production is increasing not only the protein source for the local demand but also the use of antimicrobials in these regions. During the last decades, numerous veterinary antibiotics (VAs) have been used globally as growth promoters and therapeutic agents in livestock production because of their positive effects (Arikan et al. 2007). VAs are used in poultry practice to prevent and control diseases and assist in combating stress due to environmental changes and in management practices (Pavlov et al. 2008). Antimicrobial agents are among the commonly used drugs in poultry production. In the USA, for example, the use of VAs as feeding supplements has

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increased by 109-fold from 1950 to 2004 (Arikan et al. 2009), and 60 to 80 % were used for non-therapeutic purposes (Mellon et al. 2001).

In Egypt, excessive and incorrect use of antimicrobials in veterinary medicine plays a key role in the spread of antibiotic-resistant bacteria, especially enteric pathogens (*Escherichia coli* and *Salmonella* species), that increasingly threatens the successful treatment of infectious diseases (Anderson 2003). On the other hand, many studies have shown that antibiotics administered to livestock were usually excreted without metabolism (Halling-Sørensen et al. 1998). Residues of VAs can enter the environment with the excreta of treated chickens when poultry litter is spread on agricultural land to fertilize it. Soil, thus, is the environmental compartment primarily exposed (Spaepen et al. 1997). Consequently, the active ingredients reach the upper soil layer where they either may accumulate, be rinsed off into surface waters, or leach to groundwater where they can impact both human and environmental health (Boxall et al. 2003). Currently, there is no legislation in the European Union for limits of antibiotics in soils.

Recently, the safety of VAs in the environment has become a matter of increasing public scrutiny and legal requirements. Therefore, the residues of the veterinary antibiotics in poultry farm environmental samples were taken into account in our research work. In Egypt, there have been many studies on the prevalence of antibiotic-resistant bacteria and antibiotic residues in food of poultry origin including meat and table eggs (Okerman et al. 2007; Mahmoud and Mohsen 2008); however, little is known about the prevalence and residues in environmental samples.

The aims of the present cross-sectional study were, therefore, to provide information on the prevalence and sources of key pathogens in farms, to provide data for risk assessment, and to determine the sensitivity of isolates to commonly used antimicrobial agents in the poultry industry in Egypt. Moreover, a questionnaire was structured to reflect the rate of awareness to VAs health hazards used in poultry farming. Finally, the residues and potential risks of VAs were evaluated to understand whether the poultry litter poses risks to the environment when they were introduced into fields by agricultural practices.

Materials and methods

Sample collection

The study was carried out in Sharkia Governorate which has one of the major sources of animal protein, the poultry sector, in Egypt. Out of 13 agricultural poultry sector zones in Sharkia district, four (Zagazig, Bilbeis, Minya Al Qamh, and Mashtool) were selected by simple random sampling method. Multi-stage random sampling technique was used to select farms per zone. The research was carried out in two stages: questionnaire administration and cross-sectional study.

The survey

Structured questionnaires were administered to 75 poultry farmers that represent the four investigated districts. The questionnaires were designed to assess the level of awareness of legislation guiding and guarding the use of veterinary antimicrobials with particular reference to tetracycline and ciprofloxacin. The questionnaires, for example, include pattern and frequency distribution of VAs usage in poultry farms, nature of drug preparations, staff administrating drugs, mode of administration, etc.

The cross-sectional study

A total of 225 litter, bird dropping, and water samples were collected from 75 broiler poultry farms during the period between December 2011 and September 2012. From each farm, pooled samples of litter, bird dropping, and water were taken. The litter sample was collected in a zigzag manner from different areas of the farm floor, then pooled to form one uniform sample, and packed separately in sterile plastic bags. Meanwhile, water samples were collected in 150-ml sterile glass bottles from drinkers in front of birds. Near the end of the rearing period, bird dropping samples were freshly collected from the litter surface and placed on sterile buffered peptone water. After the samples were mixed properly, they were lyophilized and sieved through a 2 mm sieve before further handling. Samples were immediately transported under cooled conditions to the laboratory and stored in the dark at 4 °C prior to analysis.

Bacterial isolation

Salmonella was isolated according to a standard method described in ISO 6579 (2002), and an appropriate method was used to isolate *E. coli*. Twenty-five grams from each sample was homogenized well with 225 ml buffered peptone water and incubated for 24 h at 37 °C for pre-enrichment. For isolation of *Salmonella*, 0.1 ml from each pre-enriched broth was transferred to tubes containing 10 ml Rappaport–Vassiliadis selective enrichment broth (Oxoid CM699) and incubated at 41 °C for 24 h. The broth cultures were then streaked on xylose lysine desoxycholate (XLD) agar (Oxoid CM469) and incubated at 37 °C for 24 h. Typical colonies were chosen for confirmatory biochemical tests and serotyped using the Kauffmann–White typing scheme by slide agglutination with standard antisera (Murex Biotech Ltd., Dartford, England, and Dade-Behring Gmbh, Marburg, Germany).

For *E. coli* isolation, 1 ml of pre-enrichment broth was transferred to 10 ml MacConkey broth (Oxoid CM5a) and incubated at 37 °C for 24 h for enrichment. Then, a loopful was streaked on Eosine methylene blue (EMB) agar (Oxoid CM69) and incubated at 37 °C for 24 h. Typical *E. coli* colonies were confirmed with a biochemical identification test. All isolates identified as *E. coli* species were serotyped. Serotyping was carried out by a national reference laboratory (Animal Health Research Institute, Doki, Giza, Egypt) using published methods.

Antimicrobial susceptibility testing

All recovered isolates of *Salmonella enterica* and *E. coli* were tested for their antimicrobial susceptibility using the disc diffusion method on Mueller–Hinton agar (Oxoid 337) according to the standard procedure of the Clinical and Laboratory Standards Institute (CLSI 2006). The antibiotics used in this study were ampicillin (10 mg), amoxicillin (25 mg), enrofloxacin (10 mg), gentamicin (10 mg), erythromycin (15 mg), ciprofloxacin (5 mg), neomycin (30 mg), trimethoprim–sulfamethoxazole (1.25/23.75 mg), chloramphenicol (30 mg), and tetracycline (30 mg). All antibiotic discs were provided by bioMerieux, F6980 Marcy l'Etoile, France. After incubating the inoculated plate aerobically at 37°C for 18–24 h in an aerobic atmosphere, the susceptibility of the isolates to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *E. coli* ATCC 35218 were used as quality control strains.

Screening of antibiotic residues

The determination of antibiotic residues was done in a high-performance liquid chromatography (HPLC) apparatus (a constant liquid chromatography pump, an autosampler, a wavelength UV detector set, ChemStation software; Agilent Thermo Scientific Co., USA) to analyze tetracycline and ciprofloxacin residues in samples that harbored *E. coli* phenotypically resistant to tetracycline and/or ciprofloxacin.

Sample extraction

For determination of tetracycline residues, frozen samples were thawed; then, 2 g of each sample was cut into very small pieces, ground into fine particles using a Sartorius mincer, and consequently homogenized in a blender for 2 min, and 0.1 ml citric acid was added. To this mixture, 1 ml nitric acid (30 %), 4 ml methanol (HPLC grade), and 1 ml deionized water were added, respectively. The suspension with solid particles was placed in a vortex, kept in an ultrasonic bath for 15 min, and centrifuged for 10 min at 5300 rpm. After filtration through a 0.45-μm nylon filter, 20 μl of solution was injected into HPLC for analysis according to Senyuva et al. (2000).

For the detection of ciprofloxacin residues, 2 g of each thawed frozen sample was cut into very small pieces and ground into fine powder using the Sartorius mincer. After that, it was homogenized in a blender for 2 min and then 8 ml citric acid was added. The suspension with solid particles was placed in a vortex for ground mixing for 1 min, then rotary agitated for 10 min, and centrifuged for 5 min at 14,000 rpm. After filtration through a 0.45-μm nylon filter, 25 μl of solution was injected into HPLC for analysis according to Gigososa et al. (2000).

Chromatographic condition and calibration curve

The separation was done on a Hypersil GOLD C18 (10 μm, 100×4.6 mm) column with mobile phase composed of methanol (HPLC grade) and formic acid 0.1 % using a gradient method with a flow rate of 1.5 ml min^{-1}

at 25 °C and 0.3 ml min^{-1} at 25 °C for tetracycline and ciprofloxacin, respectively. Detection was performed with a UV detector set at 350 and 294 nm wavelengths for tetracycline and ciprofloxacin, respectively. Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the software (ChemStation). The detection parameters for antibiotic residues are summarized in Table 1.

The concentration of antibiotic residue in the samples was calculated with reference to calibration curves that were prepared from work solutions of tetracycline and ciprofloxacin using concentrations of 0.01, 0.1, 1.0, 2.5, 5, and 10 mg l^{-1} in methanol eluent. For the preparation of the work solutions, tetracycline hydrochloride (Sigma-Aldrich, Inc., St. Louis, USA) and ciprofloxacin (Bayer Pharmaceuticals, West Haven, CT, USA) stock solutions (1 μg l^{-1}) were diluted to various concentrations as previously mentioned. The detection limit for both was 0.01 ppm, while the retention time was 3.7 and 7.15 min for tetracycline and ciprofloxacin, respectively.

Method validation

Although the liquid chromatography (LC–MS–MS) method was found to be more sensitive than HPLC with the UV detector method, we used the latter for the detection of VAs based on the following two considerations: firstly, this study aimed to investigate the VA residues in poultry litter from poultry plant operations in which most residues may be high enough to be detected by HPLC; secondly, the expected detection limitation in this study is 100 μg kg^{-1} which is the critical concentration for further risk assessment, and it could be realized by HPLC. Furthermore, in essence where plasma concentrations are high and rapid turnaround of data is not required, the use of the LC–UV is more than adequate.

Quality assurance measures including blanks; litter, water, and dropping-spiked recovery; and experimental repeatability and reproducibility were conducted to ensure the accuracy and precision of this method. Blanks were employed to quantify the concentrations of antibiotics in solution. Calibration curves were established with six points along the range of 0.01–10 mg kg^{-1}. The correlation coefficients of the objectives were all above 0.999.

Spiked samples were analyzed in four replicates. The recovery of individual antibiotics was calculated by dividing the detected concentration by the spiked concentrations in the samples. The average recovery was 89, 99, and 93 % for tetracycline and 85, 98, and 90 % for ciprofloxacin in litter, water, and bird dropping, respectively. RSDs and intra-days and inter-days for tetracycline and ciprofloxacin were 3 and 3.6 % and 4.2 and 3.9 %, respectively.

Hazard risk assessment

The ecological risk of VAs in broiler chicken litter to soil microorganisms was assessed by the value of a hazard quotient (HQ) (Eq. 1) (Park and Choi 2008). The predicted environmental concentrations (PECs) of antibiotics in soil (milligrams per kilogram) were calculated according to the method by Spaepen et al. (1997) (Eq. 2). The predicted no-effect concentrations (PNECs) of antibiotics in soil (milligrams per kilogram or milligrams per liter) were calculated using Eq. (3). If the value of the hazard quotient is below 1 unit, no ecological risks are expected; otherwise, potential ecological risks may be observed.

$$\mathrm{HQ} = \mathrm{PECs} / \mathrm{PNECs} \quad (1)$$

$$\mathrm{PECs} = (M \times C) / (100 \times D) + M \quad (2)$$

$$\mathrm{PNECs} = \mathrm{TOXs} / \mathrm{AFs} \quad (3)$$

where M is the amount of broiler chicken litter applied in farmland annually (kilograms per hectare) which is indirectly calculated by determining the limit rates for nitrogen (M_N, kilograms excreta per hectare per year—nitrogen): $M_N = A_N / P_N \times P_E$

where A_N, kilograms N per hectare per year=170; P_N, kilograms N per place per year=0.21; and P_E, the yearly manure output=37.2 kg place year (Spaepen et al. 1997).

C is the current concentration of veterinary antibiotics in broiler litter (milligrams per kilogram) and D is the mass of the top 0–20 cm of soil in farmland (kilograms per hectare). For this study, a soil depth of 5 cm with a density of 2.65 kg m^{-3} was used. As in the procedure presented by Spaepen et al. (1997), the weight of this soil (W, kilograms per hectare) is calculated using the soil volume (V, cubic meters per hectare) and bulk density (ρ, kilograms per cubic meter). The volume of the top 5 cm is calculated by converting centimeter into meter and hectare into square meter, that

Table 1 Detection parameters of analyzed antibiotics

Project	Parameter
HPLC	Agilent, Thermo Scientific, USA
Detector	UV
Wavelength	350 and 294 nm
Column	Hypersil GOLD C18 (Thermo Fisher Scientific) 100×4.6 mm, 10 μm
Column temperature	25 °C
Flow rate	1.5 and 0.3 ml min^{-1}
Mobile phase	Mobile phase A, methanol
	Mobile phase B, 0.1 % formic acid
Gradient elution	0 min, 10 % A
	18 min, 37 % A
	20 min, 80 % A
	22 min, 80 % A
	25 min, 10 % A

is, V=500. The weight of the soil is then described by the relation: $W\mathrm{s}=V\times\rho$ (Kelly et al. 2003).

TOX (milligrams per kilogram or milligrams per liter) is the toxicity of antibiotics to soil microorganisms. It can be substituted with the lowest median effective concentration (EC_{50}) or pollution-induced community tolerance. The values of median effective concentration were adopted according to studies by Thiele-Bruhn and Beck (2005). AFs are the assessment factors. The value is 1000 if the risk assessment is conducted by an acute toxicity test or 100 if based on a chronic toxicity test (EMEA 2006).

Since median effective concentrations are currently only available for six of the antibiotics, between which ciprofloxacin is not located, the potential risks of only tetracycline antibiotic were estimated. Since the risks were estimated by acute toxicity tests, assessment factors of 1000 were used here.

Results and discussion

Bacteriological examination

Of the 225 broiler chicken farm samples, 179 (79.5 %) samples for each of litter (66/75), drinking water (46/75), and bird dropping (67/75) were positive for *E. coli* as shown in Table 2. The most common isolated *E. coli* serotype was O142:K86 (20.1 %), followed by O125:K70 (13.95), O91:K and untypable (12.8 %), and O119:K69 (7.8 %). Meanwhile, *S. enterica* species was isolated in 7 (3.1 %) of 225 samples; 3 and 4 isolates were recovered from litter and bird dropping samples, respectively. No *Salmonella* was detected from water of investigated poultry farms (n=75) (Table 2). *S. enterica* serotypes emek and enteritidis were recovered in a percentage of 85.7 and 14.3 %, respectively. The results demonstrated that poultry farms in Egypt were heavily contaminated with enteric bacteria particularly pathogenic and non-pathogenic *E. coli* serotypes. The detection frequency of *E. coli* was higher than other prevailing studies in Egypt (Draz et al. 1996). This was not a surprise since the organism is part of the normal flora of the intestinal tracts of poultry; however, the recovery of *E. coli* in high prevalence from farm drinking water indicates unhygienic conditions.

Although *Salmonella* spp. prevalence from poultry farms was low, the detected serovars pose a significant public health threat because they are indicative that broiler poultry farms in Egypt harbor zoonotic organisms that have the potential of entering the food chain. Herikstad et al. (2002) considered *S. enterica* serovar enteritidis is the most common species of *Salmonella* isolated from humans worldwide. Furthermore, Hang'ombe et al. (1999) isolated *S. enteritidis* from chicken carcasses processed for human consumption and indicated that *S. enterica* serovar enteritidis are potential sources of infection to humans. Moreover, *S.* emek was one of the most common *Salmonella* serotypes in the poultry environment (Vo et al. 2006).

Table 2 Prevalence and serotypes of *E. coli* and *Salmonella* recovered from broiler chicken farms

Isolated bacteria	Source of samples	Prevalence %	Serotype	Prevalence % (no. of samples positive for that serotype)
Escherichia coli	Litter	88 (66/75)	O125:K70	19.7 (13/66)
			O142:K86	19.7 (13/66)
			O86:K71	19.7 (13/66)
			O126:K71	19.7 (13/66)
			O119:K69	21.2 (14/66)
	Drinking water	61.3 (46/75)	O142:K86	23.9 (11/46)
			O125:K70	26.1 (12/46)
			Untypable	50 (23/46)
	Bird dropping	89.3 (67/75)	O142:K86	16.4 (11/67)
			O91:K	34.3 (23/67)
			O127:K63	16.4 (11/67)
			O124:K72	16.4 (11/67)
			O25:K11	16.4 (11/67)
Salmonella spp.	Litter	4 (3/75)	*S.* emek	75 (3/4)
			S. enteritidis	25 (1/4)
	Drinking water	0.0 (0/75)		
	Bird dropping	5.3 (4/75)	*S.* emek	100 (3/3)

Hence, it is imperative that disease control strategies should not only focus on reducing the occurrence of bacterial infections in poultry but should also include the need to reduce the threat of zoonotic pathogens infecting humans.

Susceptibility testing

Prevalence of resistance of *E. coli* (n=179) and *Salmonella* spp. (n=7) isolated from broiler poultry farm samples for the ten VAs tested indicated that *E. coli* serogroups had the highest resistance to ampicillin (97.2 %), followed by amoxicillin (94.9 %), erythromycin (89.4 %), sulfamethoxazole–trimethoprim (86.6 %), tetracycline and neomycin (78.2 %), and chloramphenicol (72.6 %), but resistance to gentamicin and enrofloxacin (61.4 %), and ciprofloxacin (59.7 %) was the lowest (Table 3). *Salmonella* isolate resistance patterns to all tested antimicrobials had the nearly similar levels of resistance compared to those of *E. coli* serogroups as serovars exhibited the highest resistance to ampicillin, followed by amoxicillin, tetracycline, and sulfamethoxazole–trimethoprim. However, the lowest resistance was recorded against ciprofloxacin and enrofloxacin (Table 3).

Unfortunately, it is obvious that almost all *E. coli* and *S. enterica* isolates were resistant to more than three classes of antibiotics (multi-drug resistant), which may be attributed to the misuse of these antibiotics in veterinary treatment in Egypt. Besides, *E. coli* and *Salmonella* isolates were nearly similar in resistance, and this may be due to the potential transmission of resistant genes in between via plasmid trans-conjugation (Dahshan et al. 2010). The emergence and spread of antimicrobial resistance in bacteria of medical importance impose serious constraints on the options available for treatment of many infections, and this raises a concern among general practitioners and pediatricians in developing countries.

Questionnaire response

Based on the questionnaire survey of the farmers during the study, all farms were of deep litter management system and large scale (above 2000 bird capacity), and use commercial feeds. Regarding the pattern and frequency distribution of drug usage, farmers used various antimicrobial groups as all the investigated farms (100 %) use different groups of antibiotics in the same rearing cycle in the form of veterinary and human

Table 3 Prevalence of resistant *E. coli* and *Salmonella* species isolated from broiler chicken farms

Isolated bacteria	Source	Percentage of resistance (%)									
		ENR	CIP	SXT	AMX	CHL	AMP	TET	GEN	NEO	ERY
Escherichia coli	Litter	51.5 (34/66)	62.1 (41/66)	84.8 (56/66)	92.4 (61/66)	68.2 (45/66)	93.9 (62/66)	81.8 (54/66)	39.4 (26/66)	84.9 (56/66)	90.9 (60/66)
	Drinking water	63 (29/46)	58.7 (27/46)	89.1 (41/46)	97.8 (45/46)	84.8 (39/46)	97.8 (45/46)	87 (40/46)	80.4 (37/46)	82.6 (38/46)	90.3 (42/46)
	Bird dropping	70.1 (47/67)	58.2 (39/67)	86.5 (58/67)	95.5 (64/67)	68.7 (46/67)	100 (67/67)	68.7 (46/67)	70.1 (47/67)	68.7 (46/67)	86.6 (58/67)
Salmonella spp.	Litter	33.3 (1/3)	33.3 (1/3)	100 (3/3)	66.6 (2/3)	66.6 (2/3)	100 (3/3)	100 (3/3)	66.6 (2/3)	66.6 (2/3)	33.3 (1/3)
	Bird dropping	50 (2/4)	25 (1/4)	50 (2/4)	100 (4/4)	50 (2/4)	100 (4/4)	75 (3/4)	75 (3/4)	50 (2/4)	25 (1/4)

ENR enrofloxacin, *CIP* ciprofloxacin, *SXT* sulfamethoxazole–trimethoprim, *AMX* amoxicillin, *CHL* chloramphenicol, *AMP* ampicillin, *TET* tetracycline, *GEN* gentamicin, *NEO* neomycin, *ERY* erythromycin

preparations, staff administration of drugs were done by untrained personnel, lack of awareness of legislation guiding the use and marketing of veterinary drugs, and non-observance of withdrawal periods. This questionnaire depicts the true state of misuse of veterinary drugs amongst unqualified competitors in the poultry industries in Egypt, which eventually leads to high chances of antibiotic resistance and residue occurrences on many farms. These findings concurred with a previous report that stated chances of residue occurrence are higher when drugs are wrongly administered outside the recommendation of experts (Van Dresser and Wilcke 1989). Therefore, antibiotic residues were assessed to provide some preliminary information on the occurrence of VA residues in broiler poultry farms in Egypt as there is scanty number of studies related to VA residues in poultry farm environmental samples.

Veterinary antibiotic residues in poultry litter

From ten VAs that revealed resistance, only two (tetracycline and ciprofloxacin) were assessed for residues. As tetracycline is one of the most widely used antimicrobial drug groups in poultry farming, all the farms (100 %) screened use tetracycline as it is used as both antimicrobial and anticoccidial agent. Moreover, tetracycline commercial preparations are utilized extensively in egg production because it is believed to enhance egg production and give brown coloration to eggshells. Concerning ciprofloxacin, the quinolones group are potent inhibitors of DNA gyrase enzyme, which is critical for DNA replication and transcription (Suto et al. 1992). The toxicity and side effects of quinolones are well established in animals and humans (Khadra et al. 2012).

In this study, the mean levels of tetracycline and ciprofloxacin antibiotic residue were found to be 2.125 and 1.401 mg/kg^{-1} in positive samples [contaminated with *E. coli* phenotypically resistant to tetracycline (n=140) and ciprofloxacin (n=107)], respectively (Table 4). However, no residues for tetracycline were detected in drinking water (n=40). Consequently, we cannot affirm that the occurrence of antibiotic-resistant bacteria in a farm sample means the existence of residues in the same positive sample. The possible reasons for high antibiotic residues in broiler poultry farms in the study area may be attributed to lack of veterinary drug control, availability of numerous trade names, and forms to the same antibiotic active principles (tetracycline, ciprofloxacin) in Egyptian markets, and also poverty plays a key role in

Table 4 Mean concentration of antibiotic residues in chicken farms

Antibiotic	Antibiotic residues[a], mean (mg kg^{-1})			Poultry samples, mean (mg kg^{-1})
	Litter	Drinking water	Bird dropping	
TET	2.394	0.0	3.981	2.125
CIP	1.378	1.954	0.871	1.401

TET tetracycline, *CIP* ciprofloxacin

[a] Residues % was estimated among samples that harbored *E. coli* resistant to TET (TET residues) and CIP (CIP residues)

the latter case because most farmers cannot afford the services of a veterinarian and so resort to treating the birds themselves.

Potential risk assessment

There are several limitations to the HQ approach as HQs are combined for substances with Subchronic Reference Doses (RfDs) based on critical effects of varying toxicological significance. Also, it will often be the case that RfDs of varying levels of confidence that include different uncertainty adjustments and modifying factors will be combined (e.g., extrapolation from animals to humans, from one exposure duration to another). Moreover, the application of the HQ equation to a number of compounds that are not expected to induce the same type of effects or that do not act by the same mechanism could overestimate the potential for effects. This possibility is generally not of concern if only one or two substances are responsible for driving the HQ above unity as in our study risk assessment. Although the HQ approach has limitations, it is still one of the baseline health risk assessment processes.

According to a recommendation in the European Union (Directive 92/18/EEC 1992), a risk assessment of applying broiler chicken litter on farmlands is necessary if the concentration of veterinary antibiotics in broiler litter is over 100 μg kg^{-1}; therefore, the study area was evaluated for the environmental risk of residual VAs (tetracycline, conc. 2394 μg kg^{-1}) in broiler chicken litter. As shown in Table 5, the results of predicted environmental concentrations, predicted no-effect concentrations, and hazard quotients for tetracycline residues in poultry litter significantly display environmental risks as it is estimated to have a hazard quotient value above 1 (1.64). Based on this study, tetracycline might be one of the dangerous VAs for soil microorganisms in Egypt. The risks of tetracyclines from poultry litter in this region should be paid special attention. On the other hand, various types of VAs and other coexisting pollutants in soils may alter the toxicity of a single pollutant. Therefore, further research is strongly recommended to simulate the behaviors of poultry litter veterinary antibiotics in an actual environmental system.

Conclusions

This study identified litter, water, and bird dropping as sources of potentially clinically significant pathogens. As there are currently no validated technologies to eliminate *Salmonella* or *E. coli* in poultry farms (Horchner et al. 2006), good farming practice should focus on reducing the risk of the dissemination of these pathogens in the farming environment. Besides, it is also very obvious that poultry farmers (without consulting a veterinarian) in the study area do not adhere to withdrawal periods of antimicrobial drugs; most times, farmers do not even bother reading the manufacturer's instructions before the use of a drug. Therefore, strict regulations must be implemented to control the usage of antibiotics in order to stop the emergence of resistant strains. Public education and awareness of the risk of misuse of antimicrobial drugs are crucial. Residues, resistance patterns, and potential risk assessment indicated that VAs pose a potential for transmission of resistance through the food chain and environmental risks on local farmland.

Table 5 EC_{50} and assessment results of tetracycline residues in litter from broiler chicken

Antibiotic	EC_{50} (mg kg^{-1})	PECs (μg kg^{-1})	PNECs (μg kg^{-1})	HQ
TET	270[a]	443.34	270.0	1.64

[a] Data cited from Thiele-Bruhn and Beck (2005)

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