Antimicrobial drug resistance at the human-animal interface in Vietnam

Nguyen, V.T.

CHAPTER 4

PREVALENCE AND RISK FACTORS FOR CARRIAGE OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* ON HOUSEHOLD AND SMALL-SCALE CHICKEN FARMS IN THE MEKONG DELTA OF VIETNAM

CHAPTER 4

Chapter 4: Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong delta of Vietnam

Nguyen Vinh Trung[1–3*], Juan J. Carrique-Mas[3,4], Ngo Thi Hoa[3,4], Ho Huynh Mai[5], Ha Thanh Tuyen[3], James I. Campbell[3,4], Nguyen Thi Nhung[3], Hoang Ngoc Nhung[3], Pham Van Minh[3], Jaap A. Wagenaar[6,7], Anita Hardon[8], Thai Quoc Hieu[5] and Constance Schultsz[1–3]

[1] Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.
[2] Department of Global Health-Amsterdam Institute for Global Health and Development, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.
[3] Oxford University Clinical Research Unit, Centre for Tropical Medicine, Ho Chi Minh City, Vietnam.
[4] Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK.
[5] Sub-Department of Animal Health, My Tho, Tien Giang, Vietnam.
[6] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
[7] Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands.
[8] Center for Social Science and Global Health, University of Amsterdam, Amsterdam, The Netherlands

J. Antimicrob. Chemother. 2015 (70) 2144-2152

CHAPTER 4

Abstract

Objectives

To describe the prevalence of antimicrobial resistance (AMR) among commensal *Escherichia coli* isolates in household and small-scale chicken farms common in southern Vietnam and to investigate the association of AMR with farming practices and antimicrobial usage.

Methods

We collected data on farming and antimicrobial usage from 208 chicken farms. *E. coli* was isolated from boot swab samples using MacConkey agar (MA) and MA with ceftazidime, nalidixic acid or gentamicin. Isolates were tested for their susceptibility against 11 antimicrobials and for extended spectrum β-lactamase production. Risk factor analyses were carried out using logistic regression, at both bacterial population and farm level.

Results

E. coli resistant against gentamicin, ciprofloxacin, and 3rd-generation cephalosporins was detected in 201 (96.6%), 191 (91.8%) and 77 (37.0%) of the farms, respectively. Of 895 *E. coli* isolates, resistance against gentamicin, ciprofloxacin, and 3rd-generation cephalosporins was detected in 178 (19.9%), 291 (32.5%) and 29 (3.2%) of the isolates, respectively. Ciprofloxacin resistance was significantly associated with quinolone (OR=2.26) and tetracycline usage (OR=1.70). ESBL-producing *E. coli* were associated with farms containing fish ponds (OR=4.82).

Conclusions

Household and small farms showed frequent antimicrobial usage associated with high prevalence of resistance against the most commonly used antimicrobials. Given the weak bio-containment, the high prevalence of resistant *E. coli* could represent a risk to the environment and humans.

Keywords: antimicrobial use, antimicrobial resistance, poultry, treatment incidence

CHAPTER 4

Introduction

Antimicrobials are extensively used in animal farming with the aim to treat and prevent animal diseases, as well as to improve growth performance [1]. The overuse of antimicrobials in food animal farming is an important factor contributing to the emergence and dissemination of antimicrobial-resistant organisms in animal production systems, and contributes at an unknown level to the overall problem of antimicrobial resistance (AMR) in human medicine [2]. The use of fluoroquinolones, aminoglycosides and 3rd-generation cephalosporins in animal farming is of particular concern, since these are among the most important antimicrobials currently available to treat serious human infections [3].

Commensal *Escherichia coli* organisms are commonly used to monitor AMR prevalence in livestock and poultry, since they reflect well the selective pressure on Gram-negative enteric bacteria [4, 5] AMR determinants present in *E. coli* that are selected or amplified in farms may spread to humans either through direct contact, consumption of meat, or indirectly through environmental pathways [6]. Furthermore, some animal-derived *E. coli* strains can also be pathogenic to humans, or may act as a donor of AMR genes to other pathogenic Enterobacteriaceae [7, 8].

A number of studies has demonstrated an overall higher prevalence of AMR among chicken *E. coli* compared to human isolates [7, 9] and have incriminated chickens as a source of fluoroquinolone-resistant, extra-intestinal pathogenic *E. coli* infections in humans [7, 10]. Because of this, the recently observed increase in plasmid-mediated resistance against fluoroquinolones among *E. coli* of chicken origin is of concern [5, 11]. Human infections with micro-organism resistant against 3rd and 4th-generation cephalosporins due to the acquisition of extended spectrum β-lactamase genes have increased rapidly worldwide since they were first described in 1989. Recent reports have shown the presence of ESBL-producing *E. coli* in poultry [12-14] and a great level of molecular similarity between ESBL-producing *E. coli* from chicken meat and humans, suggesting that chickens are a major source [15-17]. A rise in aminoglycoside resistance in Gram-negative micro-organism has been described in European and Asian countries [18]. In Vietnam antimicrobials including fluoroquinolones and aminoglycosides are extensively used in large scale pig and poultry farming [19-21] and a high prevalence of AMR against both classes of antimicrobials has been observed both in commensal and zoonotic bacteria from farms and meat [22, 23].

Vietnam is an agricultural country with around 70% of the population living in rural areas. Around 40% of households engage in poultry raising [24], and 94% of these 8 million households, has a flock size of less than 50 chickens [25]. Little is known about the prevalence of AMR in *E. coli* in such relatively small production systems, and its potential association with antimicrobial use and other farming practices. It is often assumed that, compared with larger farms, backyard farms use less antimicrobial drugs and feed their chicken more often with by-products instead of (often medicated) commercial feed. We therefore carried out a survey to investigate the prevalence of AMR in *E. coli* indicator bacteria in Vietnamese household and small chicken farms, with the aims of: (1) estimating the prevalence of resistant *E. coli* against key antimicrobials, with a focus on fluoroquinolones, aminoglycosides and 3rd -generation cephalosporins; and (2) identifying risk factors for faecal carriage of AMR *E. coli* in chickens, including demographics, management practices, as well as antimicrobial usage.

Materials and methods

Study population

With an extension of 2,481 km^2, the province of Tien Giang (Vietnam) is home to approximately 1.67 million people and 5.96 million chickens. For logistic reasons the study was conducted in 3 districts (My Tho, Cho Gao and Chau Thanh) out of the 10 in the province, as they contain 44.5% of the total chicken population of the province. The study population consisted of 208 chicken farms, equally divided into two strata according to the number of chickens per farm: ≥10-200 (‘household’ farms) and >200-2,000 (‘small’ farms, in contrast to large scale farms with >2,000 chickens). To avoid regional biases in the sampling, 34 farms from each of the 4 strata (district-farm size combinations) in Cho Gao and My Tho and 36 farms from each of the 2 strata in Chau Thanh were selected.

The number of farms to be sampled from each commune (the lower administrative unit within a district) was calculated with a probability directly proportional to the number of farms in that commune according to the Vietnamese rural, agricultural and fishery census in 2006 [26]. Farms were randomly sampled from each chosen commune. Farmers refusing to participate were replaced by the next eligible farm.

Written informed consent was obtained from all farmers prior to participation in the study. The study was approved by the Sub-Department of Animal Health (SDAH) and the Peoples’ Committee of Tien Giang Province.

Data collection

Farm visits were evenly distributed over the period March 2012 - April 2013 to avoid seasonal effects. Data on antimicrobial usage and farm management practices were collected using a structured questionnaire, which was conceived in a workshop including local facilitators, and was tested in the field prior to sampling (Supplemental Material 1). The questionnaire was aimed at the person with primary responsibility for chicken husbandry and contained both open and closed questions. This person was asked about details on administration of any antibacterial formulation from restocking until the visit date for farms applying all-in-all-out (AIAO) systems, and for a fixed period of 90 days for the remaining farms not practicing AIAO.

Data on each antibacterial formulation administered (excluding coccidiostats, antiparasitic and antifungal drugs), were gathered by SDAH staff, including the commercial name of the product, presentation and number of containers used. To facilitate farmers' recall, open discussions were initiated after inspecting the medicine cabinet for all products present containing antibacterial formulations. This approach is analogous to the medicine cabinet survey used in human medicine, which has been shown to be highly effective in obtaining information on community usage of antimicrobial drugs [27].

Sample collection

From each flock, naturally pooled chicken faeces was collected from representative sections of the chicken pens/houses using 2 (household farms) or 3 pairs (small farms) of boot swabs. For unconfined flocks, boot swab samples were collected from the areas where the chickens roosted at night. Boot swabs were used to walk at least 30 steps on areas where fresh droppings were visible. For flocks on stilts or caged flocks where it was not possible to use boot swabs, visible faecal material was collected using 2-3 hand-held gauze swabs, which were similar in size to the boot swabs, each collecting material from at least 10 different locations.

Swab samples were immediately stored at 4°C, transferred to the laboratory in Ho Chi Minh City, and cultured within 24 hours after sample collection. Both interviews and faecal sample collection were conducted by trained veterinarians from Tien Giang SDAH.

***E. coli* isolation**

A fixed volume (225 mL) of Buffered Peptone Water was added to each gauze or boot swab in a separate container and was then manually shaken. One mL from each container was pipetted and pooled into a sample. From this pooled sample, 1 mL was further diluted 1:1000 in saline solution, and 50 μL of this suspension was plated onto MacConkey agar without supplement and MacConkey agar supplemented with ceftazidime (2 mg/L) to select for isolates with reduced susceptibility against 3rd-generation cephalosporins , nalidixic acid (16 mg/L) to select for isolates with reduced susceptibility against quinolones, or gentamicin (8 mg/L) to select for isolates with reduced susceptibility against gentamicin, and incubated at 37°C overnight. From each plate the total number of suspect *E. coli* colonies was counted. A random selection of five (MacConkey agar unsupplemented) and two (MacConkey agar supplemented with antimicrobial drugs) presumptive *E. coli* colonies of different morphologies were subcultured, and identified as *E. coli* using standard biochemical tests (hydrogen sulfide production, carbohydrate fermentation, urease test, nitrate reductase test, methyl red test, motility test, indole test) and/or API 20E (BioMérieux, France). Isolates confirmed as *E. coli* were tested for their antimicrobial susceptibility.

Antimicrobial susceptibility testing

For the determination of antimicrobial susceptibility, the disk diffusion method was performed and interpreted according to breakpoints as defined by Clinical and Laboratory Standard Institute (CLSI) [28]. The following antimicrobials were tested at the given disk content: ampicillin (10 μg), ceftriaxone (30 μg), ceftazidime (30 μg), amoxicillin/clavulanic acid (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), gentamicin (10 μg), amikacin (30 μg), tetracycline (30 μg), meropenem (10 μg). Potential production of ESBLs, as indicated by resistance to ceftriaxone and/or ceftazidime and by an inhibitory effect of clavulanic acid was confirmed using a double disk diffusion test according to CLSI guidelines. Strains with an intermediate sensitive result were considered resistant. A MDR strain was defined as a strain resistant to at least three different classes of antimicrobials. A farm was defined as ‘positive’ for a resistant *E. coli* if at least one *E. coli* isolate resistant against the antimicrobial drug under study was cultured from MacConkey agar either with or without supplementation with antimicrobial drugs. Quality controls for identification and sensitivity testing were performed on a weekly basis according to CLSI guidelines.

Since all MacConkey agar plates (i.e. with or without supplementation with antimicrobial drugs) were streaked using an identical inoculum, counts of *E. coli*-like colonies on each plate were used to determine the proportion of colonies resistant against ceftazidime, gentamicin and nalidixic acid in relation to the total *E. coli* population for each farm.

Data analyses

Since the study was designed as a stratified survey with fixed number of farms in each stratum, not all study units (farms) had the same probability of being selected. The prevalence of resistance against each antimicrobial of a randomly selected isolate cultured from non-selective plates, as well as the prevalence of resistance by farm was adjusted for the stratified survey design by assigning a stratum-specific sampling weight (*Wi*) to each observation unit (either isolate or farm) using the following equation: $W_i = N_T/N_i$, where N_T is the total number of farms in the three study districts (29,106) and N_i is the number of farms in each stratum sampled (i =1…6). Standard errors were corrected to take into account potential similarities of prevalence between farms in each stratum [29].

The frequency of antimicrobial treatment was quantified by calculating treatment incidence as described by Persoons et al. [30]. Treatment incidence (TI) is defined as the number of chickens per 1000 that is treated daily with one defined daily dose (DDD) for each antimicrobial administered in each farm using the following formula:

$$\text{TI} = \frac{\text{Total amount of antimicrobial administered (mg)}}{\text{DDD (mg/kg) x Number of days at risk x Total weight of chicken on farm (kg)}}$$

Total amount of an antimicrobial administered was calculated using (1) the total consumption as reported by the farmer (i.e. number of containers of antimicrobial-containing products used), (2) the concentration of the product, and (3) the reporting usage period.

Defined animal daily dose was estimated based on the dosage mentioned in the drug's instruction leaflet. In case medication was dissolved in drinking water or feed, the dosage as indicated by the manufacturer was standardised to mg/kg chicken body weight, given that an average chicken consumes 190 mL of water and 80 g of feed per day. The average weight of one chicken was considered 1kg [31]. The Anatomical Therapeutic Chemical classification system for veterinary medicinal product (ATCvet) [32] was used for antimicrobial drug identification.

To determine risk factors associated with resistance considered of clinical importance for human medicine, we modelled the probability of a randomly selected *E. coli* isolate from any given farm for the following three outcomes: (1) resistance against ciprofloxacin; (2) resistance against gentamicin; and (3) MDR. This was carried out by building hierarchical generalized linear mixed regression models with the term 'farm' modelled as a random effect.

For the outcome 'resistance against 3rd-generation cephalosporins', where we observed a very low probability of resistance amongst individual randomly selected *E. coli* isolates (3.2%), culture results from supplemented and un-supplemented plates were combined and standard logistic regression models were built to model the probability of presence of resistant strains at the farm.

To build each model, a total of 42 variables were first tested in univariable analyses including factors describing the farms (production type, size, presence of other animals), farmer demographic factors, husbandry factors and antimicrobial usage (see Supplemental Material 2 for all variables included). Variables were considered as candidate for multivariable analysis based on their biological plausibility and p-value <0.15 in the univariable analyses. Candidate variables were ranked by their degree of significance and were included in the models starting with the most significant ones using a step-wise forward approach [33]. In the final multivariable models, variables were retained if their p-value was < 0.05. All interactions between all significant variables in the model were assessed.

All statistical analyses were performed using the packages *epicalc* and *survey* with R statistical software (http://www.r-project.org).

Results

Description of farm demographic and management factors

Of 104 household farms, 76.0% raised chickens for meat, whereas 23.1% raised chickens with a mixed-purpose (meat and eggs). In contrast, 60.6% of 104 small farms raised egg laying flocks, and most of the remainder 38.5% raised meat chickens (Table 1). Confinement of chickens in pens or houses for 24 hours per day was more common in small farms compared with household farms (89.4% versus 2.0%, respectively) (p<0.001). The percentage of small farms that used commercial feed (99.0%) was greater than the percentage of household farms that followed this practice (70.2%) (p<0.001).

Table 1. Characteristics of 208 chicken farms in Tien Giang province, Vietnam studied between March 2012 and May 2013.

Variable		Household farms (N=104)	Small farms (N=104)
Age of farm manager (years) (median) (IQR)		46 (40-55)	43 (37-52)
Male farm manager (No. farms) (%)		59 (56.7%)	77 (74.0%)
Level of education attained (No. farms) (%)			
	Up to primary school	38 (36.5%)	18 (17.3%)
	Secondary school	40 (38.5%)	54 (51.9%)
	Higher	26 (25.0%)	32 (30.8%)
No. chickens (median) (IQR)		75 (63-120)	1,500 (1,000-1,900)
Production type			
	Meat	79 (76.0%)	40 (38.5%)
	Eggs	1 (1.0%)	63 (60.6%)
Mixed purpose		24 (23.1%)	1 (1.0%)
Age of chickens (weeks) (median) (IQR)		15 (8 - 20)	20 (8-32)
All-in-all-out system (No. farms) (%)		32 (30.8%)	68 (65.4%)
Chickens confined in pen/house 24h per day (No. farms) (%)		2 (2.0%)	93 (89.4%)
Source of day-old chickens (No. farms) (%)			
	Hatched on farm	59 (58.4%)	10 (11.2%)
	Local hatchery	23 (22.8%)	19 (21.3%)
	Company hatchery	8 (7.9%)	59 (66.3%)
	Other	11 (10.9%)	1 (1.1%)
Presence of animals other than chickens (No. farms) (%)		103 (99.0%)	97 (93.3%)
Duck(s)		47 (45.2%)	27 (26.0%)
Pig(s)		54 (51.9%)	42 (40.4%)
Cattle/buffalo(s)		22 (21.2%)	15 (14.4%)
Dog(s)		97 (93.3%)	83 (79.8%)
Cat(s)		58 (55.8%)	54 (51.9%)
Fish /fish pond(s)		65 (62.5%)	54 (51.9%)
Change shoes/boot before entering pen/house (No. farms) (%)		53 (51.0%)	90 (86.5%)
Foot bath/foot dip at entrance (No. farms) (%)		43 (41.3%)	82 (78.8%)
Used commercial feed (No. farms) (%)		73 (70.2%)	103 (99.0%)
Used of antimicrobials (No. farms) (%)		49 (47.1%)	72 (69.2%)

IQR: Interquartile range

Prevalence of antimicrobial resistance in E. coli isolates

A total of 895 *E. coli* isolates were recovered from un-supplemented MacConkey agar. The crude (unadjusted) and adjusted prevalence of resistance in *E. coli* isolates are presented in Table 2. Among these randomly selected *E. coli* isolates, the adjusted prevalence of resistance against ciprofloxacin was 24.2% (Table 2). The adjusted prevalence of resistance against gentamicin was 15.0% and against 'any 3rd-generation cephalosporin' (ceftazidime and/or ceftriaxone) was 3.1% (Table 2). A total of 81.3% of isolates were multidrug resistant (Table 2).

Table 2. Prevalence of antimicrobial resistance in *E. coli* isolates and in chicken farms without and with sampling adjustment in Tien Giang province, Vietnam.

Antimicrobial	*E. coli* isolates[a] (N=895)		Farms[b] (N=208)	
	Prevalence of resistance (%)	Adjusted Prevalence (%) [95% CI]	Prevalence of resistance (%)	Adjusted Prevalence (%) [95% CI]
Tetracycline	93.4	91.1 (88.4 - 93.7)	100	100 (100 - 100)
Trimethoprim-sulphamethoxazole	69.7	67.0 (62.7 - 71.3)	100	100 (100 - 100)
Chloramphenicol	68.1	61.2 (57.1 - 65.4)	99.0	100 (99.9 - 100)
Gentamicin	19.9	15.0 (11.8 - 18.1)	96.6	98.2 (95.0 - 100)
Amikacin	5.4	5.4 (3.5 - 7.4)	22.1	22.3 (13.1 - 31.5)
Ciprofloxacin	32.5	24.2 (20.3 - 28.1)	91.8	92.8 (87.2 - 98.4)
Ampicillin	86.0	83.2 (79.5 - 87.0)	100	100 (100 - 100)
Amoxicilin/clavulanic acid	47.9	44.2 (39.6 - 48.9)	95.7	95.0 (89.7 - 100)
Ceftazidime	2.0	1.9 (0.4 - 3.5)	31.2	44.2 (33.1 - 55.3)
Ceftriaxone	2.5	2.2 (0.7 - 3.7)	35.1	44.6 (33.5 - 55.7)
3rd-generation cephalosporins[c]	3.2	3.1 (1.3 - 4.9)	37.0	45.9 (34.8 - 57.0)
ESBL- confirmed	0.2	0.4 (0 - 1.1)	14.9	20.6 (11.5 - 29.7)
Meropenem	0	0	0	0
Multidrug resistant[d]	85.3	81.3 (77.8 - 84.8)	100	100 (100 - 100)

CI: Confidence interval

[a] Prevalence of resistance among *E. coli* isolates randomly picked from un-supplemented MacConkey agar plates representing an unbiased snap shot of the *E. coli* population.

[b] Prevalence of resistance among chicken farms based on the isolation of resistant *E. coli* using selective MacConkey agar containing ceftazidim, gentamicin and nalidixic acid.

[c] 3rd-generation cephalosporins: ceftazidime and/or ceftriaxone. ESBL: extended spectrum beta-lactamase

[d] Multidrug resistant: resistant against at least three different classes of antimicrobial drugs

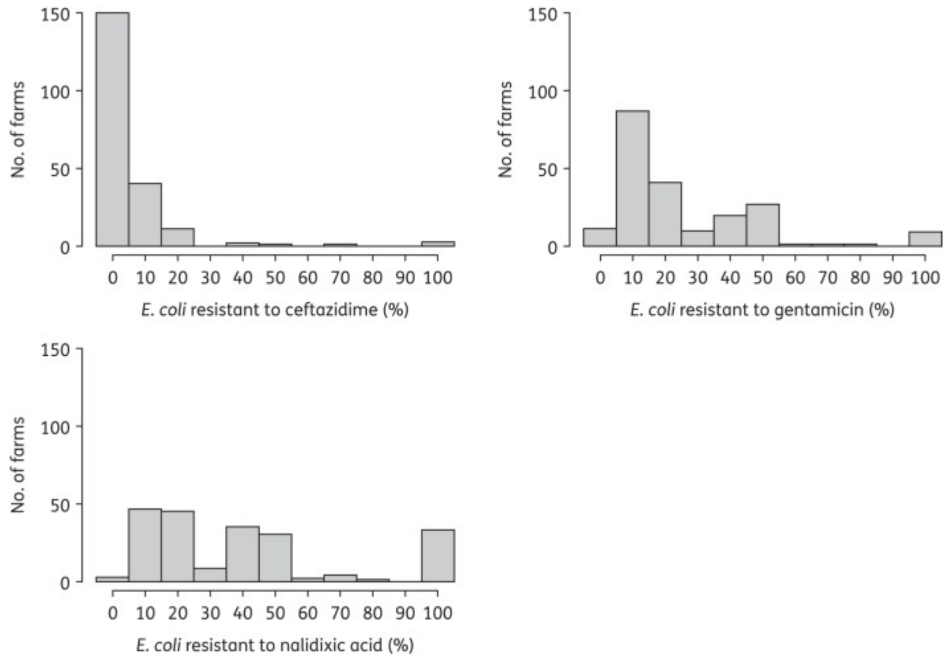
Prevalence of antimicrobial resistant E. coli in chicken farms

E. coli isolates resistant against tetracyclin, co-trimoxazole, chloramphenicol and ampicillin were detected in 100% of farms. Isolates resistant against gentamicin (98.2%), amoxicillin-clavulanic acid (95.0%), and ciprofloxacin (92.8%) were also prevalent at most farms whereas isolates resistant against ceftriaxone (44.6%), ceftazidime (44.2%), and amikacin (22.3%) were less common. From 20.6% of farms at least one ESBL-producing *E. coli* isolate was recovered. MDR *E. coli* isolates were identified in all farms (Table 2)

Proportion of E. coli isolates showing resistance by farms

The proportion of *E. coli* isolates resistant against ceftazidime, gentamicin and nalidixic acid in relation to the total *E. coli* population in each farm was estimated and was depicted in Figure 1. Gentamicin and nalidixic acid resistant colonies accounted for 100% of *E. coli* like colonies in 9 (4.3%) and 32 (15.4%) farms, respectively.

Figure 1. Distribution of the percentage of *E. coli* isolates resistant against ceftazidime, gentamicin and nalidixic acid, across all farms (N=208)



Antimicrobial usage

Treatment incidences of different classes of antimicrobial drugs are shown in Table 3. The mean treatment incidence was highest for tetracyclines (90.8) followed by macrolides (73.3), penicillins (52.1) and polymyxins (51.3) (Table 3). The treatment incidence for overall antimicrobial drug consumption was 370.6, meaning that on average per day 371 chickens out of 1000 were treated with one defined daily dose of an antimicrobial drug.

Risk factors analyses

The use of quinolones (OR=2.26) and tetracyclines (OR=1.70) was significantly associated with ciprofloxacin resistance in *E. coli* isolates (Table 4). Small farm size and farming strategies including the use of commercial feed, AIAO system and change of shoes/boots practice, were all associated with ciprofloxacin resistance but these associations were not independent (Table 4). We observed significant interactions between the size of the farm and change shoes/boot practice (OR=0.22) as well as between the usage of commercial feed and AIAO practice (OR=10.99).

Table 3. Treatment incidence of different classes of antimicrobial drugs in household and small-scale chicken farms in Tien Giang province, Vietnam (N=208)

Class of antimicrobial drug[a]	Name of antimicrobial drug	No. of farms using antimicrobial	Mean treatment incidence	Standard deviation
Tetracyclines	Docycycline, oxytetracycline, tetracycline	52	90.8	608.9
Macrolides	Tylosin, tilmicosin, erythromycin, spiramycin	40	73.3	582.0
Polymyxins	Colistin	39	51.3	234.2
Penicillins	Ampicillin, amoxicillin	33	52.1	383.1
Quinolones	Flumequine, oxolinic acid, norfloxacin, enrofloxacin	19	44.3	304.9
Aminoglycosides	Neomycine, gentamicin, apramycin, streptomycin	15	8.0	40.7
Amphenicols	Florfenicol, thiamphenicol	13	6.4	54.2
Sulfonamides	Sulfamethoxazole, sulphadimidine, sulphadimetoxine, sulphadimerazine	10	15.5	140.6
Lincosamides	Lincomycin	4	8.5	81.9
Spectinomycin	Spectinomycin	4	10.0	85.0
Trimethoprim	Trimethoprim	2	0.3	2.9
Pleuromutilins	Tiamulin	1	0.1	1.0
All classes	All antimicrobials	121	370.6	1447.4

[a] Classes were based on ATCvet classification

Lincosamides (OR=4.47) and tetracyclines (OR=1.99) usage were associated with resistance against gentamicin in *E. coli* isolates. In addition, farming strategies, including change of shoes/boot practice (OR=2.41), the purchase of day-old chicken from other sources than industrial hatchery companies (local hatcheries, markets, neighbor etc.) (OR=4.93), and raising chickens for meat or mixed (meat and egg) but not for egg laying only purpose (OR=9.88 and OR=5.03, respectively) were associated with isolation of gentamicin resistant *E. coli*. A high density of chicken (number of chickens per square meter) was associated with both gentamicin resistance and MDR. We observed a 32% and 28% increase in the odds of isolating gentamicin resistant or MDR *E. coli* respectively, for one unit increase in chicken density (chickens per square metre). The use of commercial feed was also associated with isolation of MDR *E. coli* (OR=2.49). The risk of carriage multi-drug resistance *E. coli* was decreased 4.0% for one-unit increase in the number of years of experience in chicken farming of the farmer.

The presence of fish pond(s) (OR=2.93 [95% CI, 1.11 to 7.76]) and usage of any antimicrobial drug (OR=2.80; [95% CI, 1.08 to 7.28) were associated with resistance against 3rd-generation cephalosporins in *E. coli*. The presence of fish pond(s) (OR=4.82; [95% CI, 1.27 to 18.27]), purchase of day-old chicken from other sources (i.e., local hatcheries) compared to day-old chicken from industrial hatchery companies (OR=13.02; [95% CI, 1.89 to 89.61]), and having a change of shoes/boots practice on the farm (OR=3.4; [95% CI, 0.98 to 11.81]) were associated with the presence of ESBL-producing *E. coli* on the farm.

Table 4. Risk factors for resistance against ciprofloxacin, gentamicin, and multidrug resistance in 895 randomly selected *E. coli* isolates recovered from 208 chicken farms (Tien Giang province, Vietnam).

Outcome	Variables	OR	95% CI	p-value
Ciprofloxacin resistance [a]				
	Small farm (baseline=household farm)	6.42	2.74 - 15.03	<0.001
	Use of commercial feed	1.87	1.06 - 3.30	0.032
	Change shoes/boots practice	2.43	1.44 - 4.09	<0.001
	AIAO system	0.17	0.02 - 1.28	0.086
	Use of quinolones	2.26	1.20 - 4.25	0.011
	Use of tetracyclines	1.70	1.05 - 2.76	0.031
	Interaction ‘Small farm’ and ‘Change shoes/boots’	0.22	0.09 - 0.55	0.001
	Interaction ‘Use of commercial feed’ and ‘AIAO ‘	10.99	1.38 - 87.7	0.024
Gentamicin resistance [b]				
	Use of tetracyclines	1.99	1.17 - 3.36	0.011
	Presence of cat(s)	0.44	0.24 - 0.82	0.010
	Change shoes/boots practice	2.41	1.27 - 4.59	0.007
	Day-old chickens from other sources[e]	4.93	1.22 - 19.97	0.026
	Use of lincosamides	4.74	1.18 - 18.97	0.028
	log(Density)[f]	1.32	1.02 - 1.69	0.034
	Chicken purpose (baseline= Egg laying chicken)			
	Meat chicken	9.88	5.32 - 18.33	<0.001
	Mixed chicken	5.03	1.81 - 14.01	0.002
Multidrug resistance [c,d]				
	Use of commercial feed	2.49	1.14 - 4.14	0.001
	log(Density)	1.28	1.06 - 1.54	0.008
	Year of experience in chicken farming	0.96	0.93 - 0.99	0.004

OR= Odds ratio; CI= Confidence interval; AIAO=All-in-all-out;
[a] Intercept: -2.60(SE±0.28), [b] Intercept: -5.79(SE±0.74), [c] Intercept: 1.41(SE±0.28)
[d] Resistant to at least three different classes of antimicrobial drugs; [e] Baseline = day-old chicken from industrial hatchery companies, other sources include local hatcheries, the farm and other sources. [f] No. of chickens per square metre

Discussion

This study demonstrated a very high (81.3%) prevalence of multi-drug resistant *E. coli* isolated from household and small-scale chicken farms in an unbiased study population in the Mekong Delta of Vietnam., The prevalence of resistance against both ciprofloxacin (24.2%) and gentamicin (15.0%), was substantial whilst resistance against 3rd-generation cephalosporins (3.1%) was at a much lower level. The prevalence of resistance among chicken farms based on the isolation of resistant *E. coli* using selective culture media, was very high (Table 2). Our results indicate a generally higher or similar prevalence of AMR among chicken *E. coli* isolates from Vietnam against commonly used antimicrobials (tetracycline, chloramphenicol, ampicillin, gentamicin) compared with results from industrialized countries [34-36]. Data from 7 European countries suggest a higher prevalence of ciprofloxacin resistance (57.6%), whilst data from 5 European countries indicate a higher prevalence of ceftazidime resistance (11.1%) in chickens in these countries [37]. Whilst such comparisons should be interpreted with caution because of differences in sampling methods as well as differences in breakpoints used for interpretation of susceptibility test results between studies from different regions, the high AMR prevalence observed in these backyard farms in Vietnam is striking and unexpected.

The observed high prevalence of AMR reflects the common use of antimicrobial products for therapeutic and prophylactic purpose as found in our survey on antimicrobial drug usage. Even though there was a large variation in treatment incidence between farms and between antimicrobial drugs, the treatment incidence of any antimicrobial drug usage calculated in our study (370.6) was much higher than the treatment incidence calculated for countries with industrial broiler production such as Belgium (131.8), the Netherlands (82.2) and Denmark (8.2) [30, 38] although such comparisons should be interpreted with caution given the differences in study design. In addition, most of these products were available without prescription in a pilot survey across 20 veterinary drug stores in the area (data not shown).

We found statistical associations between usage of quinolones and tetracyclines and ciprofloxacin resistance, as well as between usage of tetracyclines and lincosamides and resistance against gentamicin. Other field studies have also demonstrated that usage of quinolones selects for carriage of quinolone-resistant *E. coli* in poultry [4, 39]. The association between usage of tetracyclines and quinolone resistance may be explained by an effect of tetracycline induced mutations in the *Mar* operon resulting in over-expression of marA, which increases resistance against multiple drugs including quinolones [40]. Finally, co-selection of resistance determinants, encoded by genes located on mobile elements such as integrons, could explain the observed association between usage of tetracyclines and lincomycin, which is often formulated in combination with spectinomycin, and gentamicin resistance [41]. We acknowledge the limitations in obtaining accurate usage data derived from a cross-sectional study design. Recall biases with regards to data on usage may have introduced error with unknown impact on the observed associations. In addition, we have tried to use the treatment incidence of different antimicrobials as continuous variables in the risk factor analyses. However, we did not succeed in getting a stable model with these continuous variables and as a result we had to consider them as binary variables for the analyses. Despite these limitations, our study provides a unique view on antimicrobial drug usage and associated antimicrobial resistance in backyard chicken farms in Vietnam.

The use of commercial feed was associated with an increased risk of fluoroquinolone resistance and MDR, in agreement with a study on turkey farms in Europe [39] and reflects the fact that in Vietnam commercial poultry feed is commonly medicated with antimicrobials [42]. In this study we randomly collected 25 feed samples from 25 different chicken farms and tested these for the presence of antimicrobial agents (Premi-test, R-Biopharm AG). Antimicrobial compound(s) were detected in all feed samples (data not shown). The test, however, does not allow further identification of the antimicrobial compounds present or their concentration in the feed.

Independent of antimicrobial drug or medicated feed usage, there was mixed evidence of an association between intensification of chicken production and AMR. For example, *E. coli* isolates from household farms had clearly lower levels of ciprofloxacin resistance than isolates from small farms and an increase in density of chickens was associated with gentamicin resistance and MDR. In contrast, AIAO systems, which were more commonly observed in the larger farms, decreased risks of ciprofloxacin resistance whilst purchase of day-olds chickens from company hatcheries and the production of layer flocks were associated with lower levels of gentamicin resistance, in line with studies in Europe that reported much lower level of gentamicin resistance in layer chicken compared with broiler chickens [37].

We did not find evidence of any usage of 3rd-generation cephalosporins on any chicken farm surveyed. However, in Vietnam, cephalosporins are among the most common antimicrobial classes used in human medicine [43, 44]. It is therefore possible that transmission of resistance determinants from humans or other species (e.g. pigs) to chickens may have occurred which would explain the observed, albeit at low prevalence, ceftazidime and ceftriaxone resistance. We found that the presence of an integrated fish pond at the farm was associated with isolation of 3rd-generation cephalosporin resistant and ESBL-producing *E. coli*. We speculate that this association was related to the contact of chicken with fish pond water which would underscore the relevance of human activities for antimicrobial resistance in poultry, since a relatively high proportion of households in the rural areas of the Mekong delta do not have latrines that meet established hygienic standards in terms of construction, operation and maintenance [45]. A recent study in China suggested that the presence of ESBL-positive Enterobacteriaceae in fish farms was likely to have originated from human sewage contamination [46]. Further comparisons of isolates from humans, chickens, and fish ponds should help elucidate this relationship.

We have identified several potential risk factors for antimicrobial resistance in household and small-scale farms in southern Vietnam, which include antimicrobial usage, farm management practices, and environmental risks. Given the existing low levels of 'bio-containment' in these farms, the rare use of personal protective equipment of farming personnel when dealing with the animals, as well as the fact that there is a great degree of overlap between the farming and the household environment, the risks of transmission of AMR *E. coli* posed to both farmers and the communities living in the proximity of chicken farms are likely to be high, and need to be properly assessed in order to formulate effective strategies to limit further development of resistance to safeguard human health.

References

1. Page, S.W. and P. Gautier, *Use of antimicrobial agents in livestock.* Rev Sci Tech, 2012. **31**(1): p. 145-88.
2. Marshall, B.M. and S.B. Levy, *Food animals and antimicrobials: impacts on human health.* Clin Microbiol Rev, 2011. **24**(4): p. 718-33.
3. WHO. *Critically important antimicrobials for human medicine* 2011; Available from: http://www.who.int/foodsafety/publications/antimicrobials-third/en/.
4. da Costa, P.M., et al., *Field trial evaluating changes in prevalence and patterns of antimicrobial resistance among Escherichia coli and Enterococcus spp. isolated from growing broilers medicated with enrofloxacin, apramycin and amoxicillin.* Vet Microbiol, 2009. **139**(3-4): p. 284-92.
5. de Jong, A., B. Stephan, and P. Silley, *Fluoroquinolone resistance of Escherichia coli and Salmonella from healthy livestock and poultry in the EU.* J Appl Microbiol, 2011. **112**(2): p. 239-45.
6. Aarestrup, F.M., H.C. Wegener, and P. Collignon, *Resistance in bacteria of the food chain: epidemiology and control strategies.* Expert Rev Anti Infect Ther, 2008. **6**(5): p. 733-50.
7. Johnson, J.R., et al., *Similarity between human and chicken Escherichia coli isolates in relation to ciprofloxacin resistance status.* J Infect Dis, 2006. **194**(1): p. 71-8.
8. Hammerum, A.M. and O.E. Heuer, *Human health hazards from antimicrobial-resistant Escherichia coli of animal origin.* Clin Infect Dis, 2009. **48**(7): p. 916-21.
9. Miles, T.D., W. McLaughlin, and P.D. Brown, *Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans.* BMC Vet Res, 2006. **2**: p. 7.
10. Literak, I., et al., *Broilers as a source of quinolone-resistant and extraintestinal pathogenic Escherichia coli in the Czech Republic.* Microb Drug Resist, 2013. **19**(1): p. 57-63.
11. Huang, S.Y., et al., *Increased prevalence of plasmid-mediated quinolone resistance determinants in chicken Escherichia coli isolates from 2001 to 2007.* Foodborne Pathog Dis, 2009. **6**(10): p. 1203-9.
12. Yuan, L., et al., *Molecular characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolates from chickens in Henan Province, China.* J Med Microbiol, 2009. **58**(Pt 11): p. 1449-53.
13. Randall, L.P., et al., *Characteristics of ciprofloxacin and cephalosporin resistant Escherichia coli isolated from turkeys in Great Britain.* Br Poult Sci, 2013. **54**(1): p. 96-105.
14. Kola, A., et al., *High prevalence of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany.* J Antimicrob Chemother, 2012. **67**(11): p. 2631-4.
15. Overdevest, I., et al., *Extended-spectrum beta-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands.* Emerg Infect Dis, 2011. **17**(7): p. 1216-22.
16. Leverstein-van Hall, M.A., et al., *Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains.* Clin Microbio Infect, 2011. **17**(6): p. 873-880.

17. Depoorter, P., et al., *Assessment of human exposure to 3rd generation cephalosporin resistant E. coli (CREC) through consumption of broiler meat in Belgium.* Int J Food Microbiol, 2012. **159**(1): p. 30-8.
18. Yamane, K., et al., *Global spread of multiple aminoglycoside resistance genes.* Emerg Infect Dis, 2005. **11**(6): p. 951-3.
19. Dang, P.K., et al., *First Survey on the Use of Antibiotics in Pig and Poultry Production in the Red River Delta Region of Vietnam.* Food and Public Health 2013. **3**(5): p. 247-256.
20. CDDEP. *Situation Analysis: Antibiotic use and resistance in Vietnam.* 2010; Available from: http://www.cddep.org/sites/default/files/vn_report_web_1_8.pdf.
21. Carrique-Mas, J.J., et al., *Antimicrobial Usage in Chicken Production in the Mekong Delta of Vietnam.* Zoonoses Public Hlth, 2014. **61**(Suppl 2): p. 1-9.
22. Van, T.T., et al., *Antibiotic resistance in food-borne bacterial contaminants in Vietnam.* Appl Environ Microbiol, 2007. **73**(24): p. 7906-11.
23. Carrique-Mas, J.J., et al., *An epidemiological investigation of Campylobacter in pig and poultry farms in the Mekong delta of Vietnam.* Epidemiol Infect, 2013: p. 1-12.
24. *PRISE. A general review and a description of the poultry production in Vietnam.* 2008; Available from: http://orbi.ulg.ac.be/bitstream/2268/157619/1/2008_Review_Poultry_Prod_Vietnam.PDF.
25. Burgos S, et al., *Characterization of poultry production systems in Vietnam.* Int J of Poult Sci, 2007. **6**(10): p. 709-712.
26. *General Statistics Office of Vietnam. Results of the 2006 Rural, Agricultural and Fishery Census. Statistical Publishing House, Hanoi, Vietnam, 2007.*
27. WHO. *How to investigate the use of medicines by consumers.* 2004; Available from: http://www.who.int/drugresistance/Manual1_HowtoInvestigate.pdf.
28. *Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-first international supplement M100-S21. CLSI, Wayne, PA, USA, 2011.* 2011, Clinical and Laboratory Standards Institute.
29. Dohoo, I., W. Martyn, and H. Stryhn, *Veterinary Epidemiologic Research*. 2nd Edition ed. 2010, 2nd Edition: AVC Inc, Charlottetown, Canada.
30. Persoons, D., et al., *Antimicrobial use in Belgian broiler production.* Pre Vet Med, 2012. **105**(4): p. 320-5.
31. *MARAN 2009. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2009*. Available from: http://edepot.wur.nl/165958
32. *WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATCvet classification 2014.* Available from: http://www.whocc.no/filearchive/publications/2014_atcvet_guidelines.pdf.
33. Hosmer, D., S. Lemeshow, and R. Sturdivant, *Applied Logistic Regression*. Third ed. 2004: Wiley.
34. Tadesse, D.A., et al., *Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002.* Emerg Infect Dis, 2012. **18**(5): p. 741-9.
35. Persoons, D., et al., *Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria.* Microb Drug Resist, 2010. **16**(1): p. 67-74.
36. Ozaki, H., et al., *Antimicrobial resistance in fecal Escherichia coli isolated from growing chickens on commercial broiler farms.* Vet Microbiol, 2012. **150**(1-2): p. 132-9.
37. *EFSA and ECDC. European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food 2012.* [News] 2014; 2014/04/05:[20748]. Available from: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-in-zoonotic-and-indicator-bacteria-summary-report-2012.pdf.
38. Bondt, N., et al., *Comparing antimicrobial exposure based on sales data.* Pre Vet Med, 2013. **108**(1): p. 10-20.
39. Jones, E.M., et al., *Risk factors for antimicrobial resistance in Escherichia coli found in GB turkey flocks.* Vet Rec, 2013. **173**(17): p. 422.
40. Ruiz, C. and S.B. Levy, *Many chromosomal genes modulate MarA-mediated multidrug resistance in Escherichia coli.* Antimicrob Agents Chemother, 2010. **54**(5): p. 2125-34.
41. Toleman, M.A., P.M. Bennett, and T.R. Walsh, *ISCR elements: novel gene-capturing systems of the 21st century?* Microbio Mol Biol Rev, 2006. **70**(2): p. 296-316.
42. Dang, S.T., et al., *Impact of medicated feed on the development of antimicrobial resistance in bacteria at integrated pig-fish farms in Vietnam.* Appl Environ Microbiol, 2011. **77**(13): p. 4494-8.
43. Nguyen, K.V., et al., *Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam.* BMC Public Health, 2013. **13**: p. 1158.

44. Nga, D.T., et al., *Antibiotic sales in rural and urban pharmacies in northern Vietnam: an observational study.* BMC Pharmaco Toxicol, 2014. **15**(1): p. 6.
45. Van Minh, H., et al., *Assessing willingness to pay for improved sanitation in rural Vietnam.* Environ Health Prev Med, 2013. **18**(4): p. 275-84.
46. Jiang, H.X., et al., *Prevalence and characteristics of beta-lactamase and plasmid-mediated quinolone resistance genes in Escherichia coli isolated from farmed fish in China.* J Antimicrob Chemother, 2012. **67**(10): p. 2350-3.

CHAPTER 4

Supplementary Material 1: Questionnaire to study antimicrobial use in chicken farms in Tien Giang province, Vietnam

Name of interviewer: [________________________________]

Interview date (dd/mm/yy) : [__|__]/[__|__]/[__|__]

We are conducting a study to investigate medicines used by Tien Giang farmers for their chickens. You have received information about our study and you have agreed to participate. We would like to ask you some questions about your farm and your experience in the use of medicines for your chicken. For example, which medicines do you use and when and why. Do you agree to do the interview now?

A.	**<u>GENERAL INFORMATION</u>**	
1.	Age of farm owner/manager (years): [__|__] years	
2.	Gender:	O Male O Female
3.	Highest educational attainment:	O No schooling O Primary school O Secondary school O High school O Post-high school degree
4.	Years of experience in poultry farming:	[__|__] years
B.	**<u>PROFILE OF CHICKEN FLOCK(S):</u>**	
5.	Please provide details on chicken flock(s) present on your farm now Use a different flock number for each poultry keeping area	

<table>
<tr><th rowspan="3">Flock number</th><th rowspan="3">Chicken purpose

1-Meat chicken
2-Layer chicken
3-Mixed purpose chickens</th><th rowspan="3">Total number</th><th rowspan="3">Age

(in week/s)</th><th rowspan="3">Are your birds confined 24 hours/day in a house/pen?

0 - Unconfined
1 - Pen
2 - House</th><th rowspan="3">If confined, are they kept inside 24h/day

1 - Yes
2 - No</th><th rowspan="3">If unconfined or partly confined, do they have access to outside the farm?

1 - Yes
2 - No</th><th rowspan="3">All-in/all-out

1 - Yes
2 - No</th><th rowspan="3">No of crop/s per year</th><th rowspan="3">Expected age of depopulation or sale. If chicken are sold at different ages, indicate range

(in weeks)</th><th colspan="4">Chicken procured as</th></tr>
<tr><th colspan="2">Day-olds</th><th colspan="2">At age other than day-olds, specify age at purchase</th></tr>
<tr><th>1 - Yes
2 - No</th><th>1- Hatched in farm
2- Purchased from local hatchery
3-From company hatchery
4- Purchased from Market/ Dealer/ neighbor
9- Unknown</th><th>1 - Yes
2 - No</th><th>(in weeks)

(99 if unknown)</th></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
<tr><td>[__|__]</td><td>[__]</td><td>[__|__|__|__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td><td>[__]</td><td>[__]</td><td>[__]</td><td>[__|__]</td></tr>
</table>

6. Have any of the chickens currently present in your farm been vaccinated?
7. ○ Yes ○ No

If Yes, tick all that apply

☐ HPAI ☐ Newcastle ☐ Gumboro ☐ Infectious bronchitis

☐ Infectious encephalomyelitis ☐ Fowl ☐ Cholera ☐ Marek ☐ Other

8. Please provide details on previous chicken crop(s) – the one(s) occupying chicken pens/houses immediately before the current one(s)

Pen/House Number (use same number as in q. 5)	**No of chickens**	**Age of chickens at depopulation or sale (in weeks)**	**How many were lost due to diseases?**	**How many were lost due to other causes?**
[__|__]	[__|__|__|__]	[__|__]	[__|__|__]	[__|__|__]
[__|__]	[__|__|__|__]	[__|__]	[__|__|__]	[__|__|__]
[__|__]	[__|__|__|__]	[__|__]	[__|__|__]	[__|__|__]
[__|__]	[__|__|__|__]	[__|__]	[__|__|__]	[__|__|__]
[__|__]	[__|__|__|__]	[__|__]	[__|__|__]	[__|__|__]

9. Any there other animal species on the farm now? ○ Yes ○ No

If Yes, tick all that apply

☐ Fighting cock ☐ Duck ☐ Muscovy duck ☐ Other poultry species

☐ Pigs ☐ Cattle/buffalo ☐ Dog ☐ Cat ☐ Fish

C. <u>ANTIBIOTIC USE</u>

10. What do you do to keep your chickens healthy? Probe, anything else?
[____]
[____]

11. What products have you used to keep your chickens healthy? Do you keep them in the farm? Can we see them?
Which have you used in the current flock(s)? For continuously occupied houses or farms, describe use over the last 3 months.
If no antibiotic is used, tick this box ☐

Flock number						
Commercial Name						
Manufacturer						
Contents						
Supplier (1)						
Complete only for the current (crop)	**Presentation (2)**					
	Content per unit (eg. Grams of active compound)					
	Administration (3)					
	Total number of units used on the flock (probe, if don't remember write dk)					
	How long ago was the last administration? (days) (probe, if don't remember write dk)					
	Purpose of use (4)					
	Diseases/Problems (5)					
	Advice from (6)					
	Timing of application (7)					

Coding for products: see product list and pictures

(1) Supplier	1- Drug/feed shop; 2- Drug company/salesman; 3-Friend/neighbor; 9-Other, if applicable, specify
(2) Formulation	1- Powder; 2- Liquid
(3) Administration	1-Dissolve in drinking water; 2-Mix with feed; 3- Both dissolve in drinking water and mix with feed; 4- Injection; 5-Nose drops
(4) Purpose of use	1-Prevention; 2- Treatment; 3- Both prevention and treatment; 9- Other, specify
(5) Symptom	1-Respiratory problems; 2-Digestive problems; 3-Poor performance/Malaise; 4-High mortality; 9-Other, if applicable, write down symptoms:……………………..
(6) Advice from	1-Drug seller; 2-District veterinarian; 3-Chief of animal health worker; 4-Salesperson; 6-Friend/neighbor; 9-Other, if applicable, write down advisor ………………
(7) Timing of application	1-On arrival; 2-Before/after vaccination; 3-Changing of feed; 4-Changing of season; 5-Before selling; 6-Other, if applicable, write down timing……………………

12. Do you read the administration guidelines of the antibiotics before use?

O Always O Sometimes O Never

D. BIO-SECURITY AND CLEANING & DISINFECTION (C&D) OF CHICKEN HOUSES:

13. Ask only for enclosed chicken house/pen. Tick those that apply to your chicken flock(s):
If no chicken house/pen tick box ☐

Flock number	Ante-room	Change of boot/shoes	Foot bath/boot dip	Are outsiders allowed?
[__|__]	O Yes O No	O Yes O No	O Yes O No	O Yes O No
[__|__]	O Yes O No	O Yes O No	O Yes O No	O Yes O No
[__|__]	O Yes O No	O Yes O No	O Yes O No	O Yes O No
[__|__]	O Yes O No	O Yes O No	O Yes O No	O Yes O No
[__|__]	O Yes O No	O Yes O No	O Yes O No	O Yes O No

14. Ask only for enclosed chicken house/pen. Please describe the procedure of cleaning and disinfection in the chicken house/pen(s). Tick those that apply to your chicken flock(s):
If no chicken house/pen tick box ☐

Flock number	Type of C&D 1 - During production 2 – Terminal 3- Both 1 and 2 4-None	Mucking out 1- Yes 2-No	Washing 1- Yes 2-No	Disinfec-tion 1- Yes 2-No	What do you do with the used muck/litter/bedding? 1-Fertilize your field 2-Dispose 3-Sell it	If Dispose 1-Water way 2-Burn 3-Others
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]
[__|__]	[__]	[__]	[__]	[__]	[__]	[__]

15. Ask only for enclosed chicken house/pen. What disinfectants do you use for cleaning and disinfection of your chicken house?
If no chicken house/pen tick box ☐ If no disinfectant is used, tick this box☐.

Flock number	Commercial name of disinfectants	Dilution rate	Method /Application 1-Pressure washer 2- Sprayer 4-Backpack 5-Hose 6-Others
[__|__]			[__]
[__|__]			[__]
[__|__]			[__]
[__|__]			[__]

[__|__]			[__]

16. Wild birds seen in the farm

○ Never ○ Sometimes (1-4 times per month) ○ Often (>4 times per month)

17. Rodents seen in the farm

○ Never ○ Sometimes (1-4 times per month) ○ Often (>4 times per month)

18. Do you use any other product for disinfection? ○ Yes ○ No
a. If yes, specify: [____]

E. <u>CHICKEN FEED</u>

19. Please describe the types of feed you give to your chickens. Include feed you have given in your farm to previous flock(s) or the current chicken flock(s) only. Do not include any feed you intend to give in the future.

Flock number	Household left-overs	Uncooked rice/rice by products	Locally mixed chicken feed	Commercial feed	Others, specify
[__|__]	☐	☐	☐	☐	
[__|__]	☐	☐	☐	☐	
[__|__]	☐	☐	☐	☐	
[__|__]	☐	☐	☐	☐	
[__|__]	☐	☐	☐	☐	

20. Please provide details on commercial feed given to the current crop(s) until present.
If no commercial feed is given, tick this box ☐.

Flock number	Commercial name	Manufacturer	Presentation 1-Crumbs 2-Pellet 3-Mash	Quantity (in kg)	Given to previous crop(s) 1-Yes 2-No
[__|__]			[__]		[__]
[__|__]			[__]		[__]
[__|__]			[__]		[__]
[__|__]			[__]		[__]
[__|__]			[__]		[__]

21. Do you use other products for your chicken, for example additives to feed or drinking water?

○ Yes ○ No

If yes, list: [____]

22. Source of water for chickens (tick all that apply)

☐ Municipal supply ☐ Borehole/well ☐ Rain water

☐ Pond ☐ River/stream/canal ☐ other, specify________________

23. Distance from the farm to the closest running water sources (in meter) [__|__|__|__|__]

F.	QUESTION
24.	Do you have any questions or comments? ○ Yes ○ No [____] [____]

Thank you, this is the end of the interview.

Supplementary Material 2.1: Univariate analyses of risk factors associated with ciprofloxacin resistant *E. coli* detected among chicken *E. coli* isolated from farms.

No	Name of variable		OR	Lower 95% CI	Upper 95% CI	p-value
1	Age of farmer		0.98	0.96	1.00	0.036
2	Gender of farmer		0.60	0.39	0.92	0.018
3	No of Year Experience in chicken farming		0.96	0.93	0.99	0.003
4	Highest education of farmer					
		No schooling or primary school	1.01	0.57	1.80	0.737
		Secondary school	1.20	0.69	2.07	0.737
		Higher than secondary school	ref	ref	ref	ref
5	Small farm (baseline=household farm)		3.24	2.43	4.34	0.000
6	Chicken production type					
		Egg laying chicken	ref	ref	ref	ref
		Meat chicken	3.31	2.46	4.47	0.227
		Mixed chicken	2.92	1.79	4.75	0.227
7	Chicken confined 24h inside		3.35	1.79	6.28	0.000
8	Farm followed All-in-all-out practice		2.23	1.44	3.47	0.000
9	Location of farm					
		My Tho	ref	ref	ref	ref
		Cho Gao	1.99	1.20	3.29	0.137
		Chau Thanh	2.20	1.30	3.72	0.137
10	Chicken visited at later stage of production (baseline=early stage)		0.59	0.39	0.90	0.016
11	Farm used commercial feed		2.83	1.81	4.41	0.000
12	Farm used disinfectants		0.91	0.32	2.54	0.850
13	Farm with the presence of ante-room		0.39	0.05	3.35	0.395
14	Farm followed changing shoes/boot practice		3.17	1.94	5.20	0.000
15	Farm with the presence of footbath		2.35	1.51	3.64	0.000
16	Farm with access of outsider		1.06	0.55	2.06	0.862
17	log(Density of chicken - per square meter)		1.10	0.95	1.28	0.196
18	Rodents seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	0.82	0.53	1.25	0.702
		Often	0.82	0.18	3.72	0.702
19	Wildbird seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	1.13	0.73	1.76	0.647
		Often	0.67	0.16	2.75	0.647
20	Chicken procured as day-olds		0.61	0.20	1.90	0.395
21	Day-old chicken from other sources than industrial hatchery company		0.86	0.39	1.92	0.718
22	Presence of dog		1.69	0.80	3.55	0.169
23	Presence of duck		0.92	0.59	1.42	0.698
24	Presence of fighting cock		1.03	0.64	1.65	0.899
25	Presence of fish pond		0.69	0.45	1.06	0.090
26	Presence of cat		0.53	0.34	0.81	0.004
27	Presence of Cattle/Buffalo		1.19	0.67	2.14	0.550
28	Presence of Pigs		1.06	0.69	1.62	0.792
29	Read guideline before usage antimicrobials					
		Always	ref	ref	ref	ref
		Sometimes	1.07	0.60	1.90	0.962
		Never	1.11	0.38	3.20	0.962
30	Farm use antimicrobials (any)		2.61	1.64	4.14	0.000
31	Used aminoglycosides		0.52	0.19	1.45	0.212
32	Used amphenicols		0.83	0.16	4.41	0.830
33	Used lincosamides		2.60	1.12	6.04	0.026
34	Used macrolides		2.55	1.47	4.45	0.001
35	Used penicillins		0.53	0.23	1.25	0.147
36	Used pleuromutilins		NC	NC	NC	NC
37	Used polymyxins		0.53	0.25	1.15	0.111
38	Used quinolones		2.83	1.55	5.16	0.001
39	Used spectinomycin		2.04	0.79	5.22	0.139
40	Used sulfonamides		0.28	0.06	1.24	0.094
41	Used tetracyclines		2.25	1.43	3.56	0.001
42	Used trimethoprim		0.09	0.06	0.14	0.000

Supplementary Material 2.2: Univariate analyses of risk factors associated with ciprofloxacin resistant *E. coli* detected among chicken *E. coli* isolated from farms.

No	Name of variable	OR	Lower 95% CI	Upper 95% CI	p-value
1	Age of farmer	0.99	0.97	1.02	0.546
2	Gender of farmer	0.47	0.29	0.79	0.004
3	No of Year Experience in chicken farming	0.96	0.92	0.99	0.010
4	Highest education of farmer				
	No schooling or primary school	1.41	0.66	3.03	0.388
	Secondary school	1.62	0.74	3.56	0.388
	Higher than secondary school	ref	ref	ref	ref
5	Small farm (baseline=household farm)	2.30	1.64	3.22	0.000
6	Chicken production type				
	Egg laying chicken	ref	ref	ref	ref
	Meat chicken	4.68	3.28	6.68	0.138
	Mixed chicken	3.07	1.50	6.30	0.138
7	Chicken confined 24h inside	3.64	2.05	6.45	0.000
8	Farm followed All-in-all-out practice	1.02	0.61	1.69	0.946
9	Location of farm				
	My Tho	ref	ref	ref	ref
	Cho Gao	1.19	0.73	1.95	0.012
	Chau Thanh	0.55	0.29	1.05	0.012
10	Chicken visited at later stage of production (baseline=early stage)	0.55	0.33	0.92	0.022
11	Farm used commercial feed	1.26	0.80	1.99	0.320
12	Farm used disinfectants	0.52	0.18	1.57	0.250
13	Farm with the presence of ante-room	0.71	0.08	6.32	0.758
14	Farm followed changing shoes/boot practice	3.22	1.77	5.87	0.000
15	Farm with the presence of footbath	1.49	0.90	2.46	0.118
16	Farm with access of outsider	0.52	0.19	1.42	0.205
17	log(Density of chicken - per square meter)	1.18	1.02	1.37	0.026
18	Rodents seen in farm				
	Never	ref	ref	ref	ref
	Sometimes	0.55	0.32	0.93	0.101
	Often	1.32	0.29	5.88	0.101
19	Wildbird seen in farm				
	Never	ref	ref	ref	ref
	Sometimes	0.79	0.48	1.31	0.705
	Often	0.74	0.16	3.48	0.705
20	Chicken procured as day-olds	0.50	0.13	1.92	0.315
21	Day-old chicken from other sources than industrial hatchery company	3.96	1.08	14.52	0.038
22	Presence of dog	12.63	4.36	36.58	0.000
23	Presence of duck	0.85	0.51	1.40	0.517
24	Presence of fighting cock	0.80	0.42	1.53	0.496
25	Presence of fish pond	0.53	0.32	0.90	0.018
26	Presence of cat	0.33	0.19	0.58	0.000
27	Presence of Cattle/Buffalo	1.53	0.81	2.92	0.193
28	Presence of Pigs	1.31	0.78	2.20	0.307
29	Read guideline before usage antimicrobials				
	Always	ref	ref	ref	ref
	Sometimes	0.56	0.22	1.38	0.253
	Never	0.52	0.14	2.01	0.253
30	Farm use antimicrobials (any)	3.09	1.72	5.55	0.000
31	Used aminoglycosides	0.91	0.30	2.74	0.868
32	Used amphenicols	0.05	0.03	0.08	0.000
33	Used lincosamides	4.23	1.54	11.67	0.005
34	Used macrolides	2.66	1.49	4.74	0.001
35	Used penicillins	0.97	0.42	2.21	0.941
36	Used pleuromutilins	3.79	0.51	28.48	0.195
37	Used polymyxins	0.54	0.22	1.31	0.172
38	Used quinolones	1.62	0.69	3.78	0.269
39	Used spectinomycin	2.90	1.04	8.12	0.043
40	Used sulfonamides	0.01	0.01	0.02	0.000
41	Used tetracyclines	2.48	1.56	3.93	0.000
42	Used trimethoprim	0.05	0.01	0.20	0.000

Supplementary Material 2.3: Univariate analyses of risk factors associated with multidrug resistant *E. coli* detected among chicken *E. coli* isolated from farms.

No	Name of variable		OR	Lower 95% CI	Upper 95% CI	p-value
1	Age of farmer		1.00	0.98	1.02	0.915
2	Gender of farmer		0.61	0.37	1.00	0.049
3	No of Year Experience in chicken farming		0.95	0.92	0.97	0.000
4	Highest education of farmer					
		No schooling or primary school	0.75	0.39	1.42	0.650
		Secondary school	0.87	0.45	1.66	0.650
		Higher than secondary school	ref	ref	ref	ref
5	Small farm (baseline=household farm)		2.99	1.90	4.71	0.000
6	Chicken production type					
		Egg laying chicken	ref	ref	ref	ref
		Meat chicken	8.58	1.60	46.07	0.010
		Mixed chicken	5.02	0.90	28.05	0.010
7	Chicken confined 24h inside		2.67	0.82	8.67	0.102
8	Farm followed All-in-all-out practice		2.78	1.47	5.23	0.002
9	Location of farm					
		My Tho	ref	ref	ref	ref
		Cho Gao	1.15	0.75	1.77	0.022
		Chau Thanh	2.08	1.24	3.50	0.022
10	Chicken visited at later stage of production (baseline=early stage)		0.63	0.38	1.04	0.071
11	Farm used commercial feed		3.12	1.89	5.16	0.000
12	Farm used disinfectants		0.50	0.28	0.90	0.021
13	Farm with the presence of ante-room		1.89	0.21	17.22	0.573
14	Farm followed changing shoes/boot practice		1.11	0.70	1.76	0.671
15	Farm with the presence of footbath		1.62	0.99	2.67	0.056
16	Farm with access of outsider		1.23	0.58	2.58	0.589
17	log(Density of chicken - per square meter)		1.45	1.23	1.72	0.000
18	Rodents seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	1.84	1.12	3.00	0.014
		Often	1.96	1.26	3.05	0.014
19	Wildbird seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	1.06	0.66	1.70	0.642
		Often	1.83	0.45	7.39	0.642
20	Chicken procured as day-olds		0.39	0.05	3.07	0.371
21	Day-old chicken from other sources than industrial hatchery company		0.14	0.03	0.73	0.020
22	Presence of dog		5.62	2.35	13.43	0.000
23	Presence of duck		1.82	1.11	2.97	0.017
24	Presence of fighting cock		0.67	0.39	1.12	0.127
25	Presence of fish pond		0.65	0.40	1.05	0.076
26	Presence of cat		0.85	0.53	1.35	0.481
27	Presence of Cattle/Buffalo		0.68	0.41	1.13	0.141
28	Presence of Pigs		1.07	0.66	1.72	0.785
29	Read guideline before usage antimicrobials					
		Always	ref	ref	ref	ref
		Sometimes	0.51	0.28	0.91	0.002
		Never	0.25	0.11	0.60	0.002
30	Farm use antimicrobials (any)		1.28	0.80	2.04	0.297
31	Used aminoglycosides		4.05	0.64	25.59	0.137
32	Used amphenicols		0.25	0.09	0.64	0.004
33	Used lincosamides		5.61	0.72	43.94	0.101
34	Used macrolides		1.79	0.75	4.28	0.191
35	Used penicillins		1.67	0.83	3.35	0.149
36	Used pleuromutilins		NC	NC	NC	NC
37	Used polymyxins		0.68	0.39	1.18	0.167
38	Used quinolones		0.85	0.37	1.93	0.695
39	Used spectinomycin		NC	NC	NC	NC
40	Used sulfonamides		0.40	0.18	0.88	0.023
41	Used tetracyclines		1.48	0.76	2.90	0.250
42	Used trimethoprim		NC	NC	NC	NC

Supplementary Material 2.4: Univariate analyses of risk factors associated with 3rd generation cephalosporin resistant *E. coli* detected on farms.

No	Name of variable		OR	Lower 95% CI	Upper 95% CI	p-value
1	Age of farmer		0.99	0.96	1.03	0.652
2	Gender of farmer		1.01	0.40	2.54	0.978
3	No of Year Experience in chicken farming		0.97	0.91	1.02	0.228
4	Highest education of farmer					
		No schooling or primary school	1.31	0.42	4.09	0.543
		Secondary school	0.74	0.23	2.32	0.543
		Higher than secondary school	ref	ref	ref	ref
5	Small farm (baseline=household farm)		0.61	0.31	1.21	0.157
6	Chicken production type					
		Egg laying chicken	ref	ref	ref	ref
		Meat chicken	15.04	2.34	96.72	0.277
		Mixed chicken	16.11	2.14	121.38	0.277
7	Chicken confined 24h inside		0.70	0.19	2.55	0.591
8	Farm followed All-in-all-out practice		0.55	0.21	1.42	0.216
9	Location of farm					
		My Tho	ref	ref	ref	ref
		Cho Gao	1.29	0.49	3.37	0.659
		Chau Thanh	1.64	0.64	4.21	0.659
10	Chicken visited at later stage of production (baseline=early stage)		1.46	0.58	3.69	0.424
11	Farm used commercial feed		1.22	0.42	3.55	0.711
12	Farm used disinfectants		2.17	0.29	16.07	0.450
13	Farm with the presence of ante-room		NC	NC	NC	NC
14	Farm followed changing shoes/boot practice		1.57	0.64	3.85	0.325
15	Farm with the presence of footbath		1.35	0.54	3.34	0.520
16	Farm with access of outsider		0.76	0.20	2.86	0.691
17	log(Density of chicken - per square meter)		1.17	0.86	1.58	0.330
18	Rodents seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	1.41	0.56	3.56	0.722
		Often	1.57	0.17	15.00	0.722
19	Wildbird seen in farm					
		Never	ref	ref	ref	ref
		Sometimes	0.62	0.24	1.58	0.399
		Often	1.87	0.24	14.53	0.399
20	Chicken procured as day-olds		0.45	0.04	4.57	0.498
21	Day-old chicken from other sources than industrial hatchery company		1.75	0.35	8.67	0.493
22	Presence of dog		0.22	0.03	1.56	0.133
23	Presence of duck		1.05	0.43	2.59	0.914
24	Presence of fighting cock		1.16	0.43	3.11	0.763
25	Presence of fish pond		2.49	0.99	6.28	0.055
26	Presence of cat		0.71	0.29	1.76	0.462
27	Presence of Cattle/Buffalo		1.96	0.63	6.15	0.247
28	Presence of Pigs		0.41	0.16	1.03	0.059
29	Read guideline before usage antimicrobials					
		Always	ref	ref	ref	ref
		Sometimes	1.18	0.37	3.81	0.820
		Never	0.55	0.06	5.44	0.820
30	Farm use antimicrobials (any)		2.37	0.95	5.88	0.066
31	Used aminoglycosides		0.76	0.15	3.71	0.731
32	Used amphenicols		1.72	0.11	26.39	0.698
33	Used lincosamides		1.19	0.15	9.29	0.867
34	Used macrolides		0.65	0.16	2.69	0.550
35	Used penicillins		1.24	0.36	4.28	0.737
36	Used pleuromutilins		NC	NC	NC	NC
37	Used polymyxins		2.76	0.84	9.08	0.097
38	Used quinolones		3.14	0.68	14.58	0.146
39	Used spectinomycin		3.37	0.33	34.36	0.307
40	Used sulfonamides		2.48	0.39	15.79	0.338
41	Used tetracyclines		1.17	0.39	3.56	0.778
42	Used trimethoprim		NC	NC	NC	NC

Supplementary Material 2.5: Univariate analyses of risk factors associated with ESBL-producing *E. coli* detected on farms.

No	Name of variable	OR	Lower 95% CI	Upper 95% CI	p-value
1	Age of farmer	0.97	0.92	1.02	0.210
2	Gender of farmer	0.81	0.26	2.50	0.709
3	No of Year Experience in chicken farming	0.93	0.86	1.01	0.101
4	Highest education of farmer				
	No schooling or primary school	2.03	0.49	8.33	0.571
	Secondary school	1.23	0.28	5.42	0.571
	Higher than secondary school	ref	ref	ref	ref
5	Small farm (baseline=household farm)	0.39	0.15	1.05	0.064
6	Chicken production type				
	Egg laying chicken	ref	ref	ref	ref
	Meat chicken	23.51	2.56	215.76	0.468
	Mixed chicken	25.76	2.28	291.54	0.468
7	Chicken confined 24h inside	1.18	0.20	7.02	0.852
8	Farm followed All-in-all-out practice	0.83	0.26	2.70	0.760
9	Location of farm				
	My Tho	ref	ref	ref	ref
	Cho Gao	1.20	0.38	3.77	0.597
	Chau Thanh	0.79	0.24	2.62	0.597
10	Chicken visited at later stage of production (baseline=early stage)	1.58	0.48	5.20	0.457
11	Farm used commercial feed	0.87	0.23	3.27	0.832
12	Farm used disinfectants	5.68	0.61	52.75	0.128
13	Farm with the presence of ante-room	NC	NC	NC	NC
14	Farm followed changing shoes/boot practice	2.52	0.79	8.06	0.120
15	Farm with the presence of footbath	1.79	0.58	5.48	0.309
16	Farm with access of outsider	0.45	0.08	2.55	0.371
17	log(Density of chicken - per square meter)	0.91	0.66	1.26	0.578
18	Rodents seen in farm				
	Never	ref	ref	ref	ref
	Sometimes	0.81	0.26	2.51	0.531
	Often	0.20	0.02	2.25	0.531
19	Wildbird seen in farm				
	Never	ref	ref	ref	ref
	Sometimes	0.95	0.30	2.97	0.323
	Often	0.12	0.01	1.26	0.323
20	Chicken procured as day-olds	NC	NC	NC	NC
21	Day-old chicken from other sources than industrial hatchery company	11.40	1.65	78.95	0.015
22	Presence of dog	0.26	0.04	1.71	0.163
23	Presence of duck	0.77	0.25	2.39	0.652
24	Presence of fighting cock	1.86	0.57	6.04	0.301
25	Presence of fish pond	3.32	0.95	11.61	0.061
26	Presence of cat	0.76	0.25	2.34	0.636
27	Presence of Cattle/Buffalo	1.75	0.50	6.15	0.385
28	Presence of Pigs	0.54	0.18	1.67	0.288
29	Read guideline before usage antimicrobials				
	Always	ref	ref	ref	ref
	Sometimes	1.83	0.48	7.01	0.612
	Never	2.12	0.21	21.84	0.612
30	Farm use antimicrobials (any)	2.08	0.67	6.47	0.205
31	Used aminoglycosides	1.04	0.17	6.43	0.965
32	Used amphenicols	5.79	0.37	90.63	0.212
33	Used lincosamides	NC	NC	NC	NC
34	Used macrolides	1.33	0.26	6.85	0.732
35	Used penicillins	2.46	0.62	9.67	0.200
36	Used pleuromutilins	NC	NC	NC	NC
37	Used polymyxins	1.91	0.51	7.17	0.337
38	Used quinolones	1.33	0.24	7.44	0.742
39	Used spectinomycin	NC	NC	NC	NC
40	Used sulfonamides	4.34	0.66	28.48	0.127
41	Used tetracyclines	1.20	0.31	4.64	0.792
42	Used trimethoprim	NC	NC	NC	NC