PATTERNS OF ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* ISOLATES FROM CHICKENS IN THE PHILIPPINES

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ABSTRACT

This study investigated the patterns of antimicrobial resistance of commensal Escherichia coli in broiler chickens at slaughter. E. coli isolates were obtained from cecal contents of chickens from 40 farms at slaughter plants in Region 4A. Using the disk diffusion method the researchers determined the antimicrobial susceptibility to seven antimicrobial agents belonging to drug classes used in human therapy. Resistance was observed in 38 (95%) farms. Highest resistance levels found were to three drug classes namely, penicillins (94%), quinolones (89.4%), and tetracyclines (88.7%). Multidrug resistance (MDR) was recorded in 36 (94.7%) farms. Twenty resistance patterns were observed, 18 (90%) of which were to multiple classes involving three to six drug classes. The most common resistance pattern was to three antimicrobial classes, penicillins-quinolones- tetracyclines which was found in 6 (15.8%) of E.coli isolates from 6 farms. The high proportion of MDR to medically important antimicrobials suggests that MDR bacteria can enter the food chain and may result in widespread dissemination and transfer to humans. Hence, the use of antimicrobials in the farms must be reduced, and molecular analysis of the genes encoding for resistance is recommended.

Keywords: *Escherichia coli*; medically important antimicrobials; multidrug resistance; Philippines; poultry

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INTRODUCTION

Poultry production is one of the most important agriculture sectors in the Philippines; it contributes 11% to the gross domestic product. It is, therefore, essential to provide safe food products and to protect both public health and the poultry industry. In the last ten years, however, with the increasing use of antimicrobials to meet market demand, the poultry industry has been faced with many disease and production challenges.

Antimicrobials are used to keep animals healthy, control disease outbreaks, enhance growth and increase body weight by improving efficiency of feed utilization. Often, the whole flock is medicated via feeds and water.

This practice is common in the Philippines. The use of antimicrobials creates selection pressure in the intestinal bacteria, allowing the selection of bacteria resistant to the antimicrobials[1]. Unfortunately, resistant strains or resistance determinants from food-producing animals can be transferred to human pathogens through direct contact or indirectly through the food supply[2,3].

There is a growing concern about antimicrobial use in animal agriculture as a key factor contributing to antimicrobial-resistant infections in humans[4]. A number of studies have shown an association between the use of antimicrobials in animals and antimicrobial resistance (AMR) in humans. For example, Johnson et al.[5] reported the similarity of drug-resistant E. coli isolates from poultry and humans, suggesting that the resistant bacteria may have originated in poultry. Many of the antimicrobials used in animals are the same or similar to those for the treatment of human diseases.

In the Philippines, data about AMR in animals and animal products are insufficient. Most of the data on AMR are derived from researches conducted in universities. Information on AMR is important as evidence for policy recommendation to reduce antimicrobial use in order to contain AMR and to aid in risk assessment of antimicrobials. Thus, the present study investigated the resistance of commensal E. coli from healthy broiler chickens at slaughter and its potential impact on public health.

Materials and Methods

Sample collection

A total of 120 chickens (five chickens per farm) were randomly selected in four slaughter plants in Region 4A to collect cecal contents. The samples were individually bagged and transported on ice to the laboratory for immediate analysis.

E. coli Isolation and Identification

The isolation and identification of E. coli were done using standard method[6]. One confirmed E. coli isolate was selected for antimicrobial susceptibility testing.

Antimicrobial Susceptibility determination

Seven antimicrobial agents were used in the susceptibility testing of *E. coli* isolates (Table 1). These were cefotaxime, ampicillin, ciprofloxacin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole and tetracycline. All drugs in the panel belonged to classes that are used in human therapy. The first four drugs are critically important and the last three antimicrobials are highly important in the treatment of human infections[7].

The Kirby-Bauer disk diffusion method was used in accordance with the protocol described by the Clinical and Laboratory Standards Institute (CLSI)[8]. *E. coli* ATCC® 25922™ was used for quality control. The disk diffusion zones of inhibition around the tested antimicrobial discs were measured and interpreted based on the breakpoints set by CLSI and were also used to classify isolates into resistant, susceptible or intermediate.

Data Analysis

Data were entered in Microsoft Excel and checked for errors and inconsistencies. Percentage resistance was calculated. The Chi square test was used to determine the difference in percentage resistance.

Table 1. Antimicrobial agents used in the disk diffusion assay, concentrations and CLSI breakpoints

Drug class	Antimicrobial agents	Disk concentration (μg)	CLSI breakpoints (millimeter)		
			Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin	10	≥ 15	13-14	≤ 12
Cephalosporins (third generation)	Cefotaxime	30	≥ 26	23-25	≤ 22
Quinolones	Ciprofloxacin	5	≥ 21	16-20	≤ 15
Penicillins	Ampicillin	10	≥ 17	14-16	≤ 13
Amphenicols	Chloramphenicol	30	≥ 18	13-17	≤ 12
Tetracyclines	Tetracycline	30	≥ 15	12-14	≤ 11
Sulfonamides, dihyrodofolate reductase inhibitors and other combinations	Trimethoprim-sulfamethoxazole	25/23.75	≥ 16	11-15	≤ 10

RESULTS AND DISCUSSION

AMR is a menace to health, food safety, and the economy. It increases the duration of illness, the risk of dying and treatment costs, as well as reduces the life span of effective antimicrobials. Antimicrobial use in animals may allow the spread of resistant strains to humans through the food chain and cause resistant bacterial infections.

The present study used commensal *E. coli* as an indicator of antimicrobial load, the selection pressure exerted by antimicrobials on the gut bacteria and the extent of AMR problem in animals. In addition, this intestinal bacterium might contaminate meat and its products and thereby enter the intestinal tracts of people. *E. coli* was recovered from all 40 farms in the present study.

The broiler chickens were raised under contract growing arrangement from two integrator companies. Almost three-fourths of the study farms were under contract growing arrangement with company A and the remainder (27.5%) to company B; the difference was statistically significant (*P*=0.004).

Bacterial resistance to at least two antimicrobial classes (Fig. 1). was detected in 38 farms (95%). Highest resistance levels were found to three drug classes namely penicillins (94%), quinolones (89.4%) and tetracyclines (88.7%). Multiple drug resistance (MDR), *i.e.*, resistance to three or more classes[9] was recorded in isolates from 36 farms (94.7%). Of these, isolates from 12 farms were resistant to at least three antimicrobial classes (33.3%); 11 farms to four classes (30.6); six farms to five classes (16.7%) and seven farms to six drug classes (19.4%).

The very high farm level resistance observed in this study may reflect the extensive antimicrobial usage in the studied farms. In the Philippines, antimicrobials are widely used in broiler production for prevention, treatment and growth promotion. These are commonly provided to the whole flock in therapeutic and sub-therapeutic concentrations. Antimicrobial usage may increase the selective pressure in both pathogens and commensals [4,10]. The observed level of resistance was higher than the reports in Iran at 88%[11], Poland at 82%[12] and Germany at 68%[13]. It is also higher compared to another local study[14]. The difference in percentage resistance could be attributed to differences in antimicrobial use and usage patterns in farms or to different country policies and regulation on antimicrobial usage.

Fig.1. *E. coli* resistance profile by farms showing the wide diversity of resistance among broiler chicken farms.

Abbreviation: SDHFRIC = sulfonamides, dihydrofolate reductase inhibitors and combinations

Farms	Cephalosporin (3rd generation)	Penicillins	Quino-lones	Amino-glycosides	Amphenicols	SDHF RIC	Tetra-cyclines	No. of resistant class
2		■				■		2
4		■	■					2
3		■	■			■		3
5		■	■				■	3
6	■	■	■					3
31		■	■			■		3
12		■	■			■		3
7		■	■				■	3
9		■	■				■	3
10		■	■				■	3
13		■	■				■	3
33		■	■				■	3
8			■			■	■	3
16			■			■	■	3
11		■	■		■		■	4
32		■	■	■			■	4
15		■	■			■	■	4
18		■	■			■	■	4
34		■	■			■	■	4
35		■	■			■	■	4
38		■	■			■	■	4
21		■		■		■	■	4
37		■	■		■		■	4
39		■	■		■		■	4
40		■	■		■		■	4
14	■	■	■			■	■	5
17		■	■	■		■	■	5
20		■	■	■		■	■	5
36		■	■		■	■	■	5
19	■	■	■		■		■	5
24		■	■		■	■	■	5
22		■	■	■	■	■	■	6
23		■	■	■	■	■	■	6
25	■	■	■		■	■	■	6
26	■	■	■		■	■	■	6
27	■	■	■		■	■	■	6
28	■	■		■	■	■	■	6
29	■	■	■		■	■	■	6

The percentage of farms with MDR isolate is also extremely high and is a cause for serious concern because chickens from these farms can be potential sources of MDR-bacteria that may spread to humans or the environment. The prolonged and sub-therapeutic uses in poultry of one or several antimicrobials from different classes may explain such findings. MDRs are frequent in areas where antimicrobials are commonly given in animal production[15].

That several mechanisms of AMR exist may explain the occurrence of MDR, but among these, the presence of multidrug efflux pumps is important. For these pumps increase the expulsion of several antimicrobials out of the bacterial cell causing lower concentrations that are insufficient to effect treatment and, instead, predispose the animals to MDR.

This observation has been increasingly found in resistant isolates of clinical importance[16]. Furthermore, the co-existence of resistance genes in the mobile genetic elements (plasmids, transposons or integrons) may also be contributory to MDR. The clustering of genes in these transferrable elements may result in the transfer of all genes contained in these genetic structures even if selective pressure is directed only to a specific gene[3]. In this case, even the use of a single antimicrobial in the farms may also result in MDR.

All *E. coli* isolated from farms in company B were multidrug-resistant compared with those in company A at 92.8%; the difference, however was not statistically significant (*P*=0.3843). The observed MDR rate may indicate the high potential risk for these MDR-*E.coli* to contaminate carcasses during evisceration or further processing and be transferred to people through contact during slaughter, handling or by ingestion of contaminated meat.

Diverse resistance patterns were observed from the farms in this study. Twenty different resistance patterns were detected, 18 (90%) of which were to multiple classes. This percentage was lower compared to the 22 class resistant patterns reported in a local study[14]. The difference can be attributed to the number of antimicrobial classes that was tested being high with that of Jiao's[14].

Fig. 2. shows the resistance profile of bacteria by classes. The most common pattern is penicillin-quinolones-tetracyclines, which was observed in isolates from six (15.8%) farms. Penicillins and quinolones are critically important antimicrobials (CIAs) while tetracyclines are highly important antimicrobials (HIA) used in human medicine. These classes are also among the antimicrobials approved and are commonly used in poultry in the Philippines. Quinolones such as enrofloxacin and norfloxacin are also frequently used in broilers. The observed resistance to quinolones was higher compared to previous studies in China (67.6 to 77.1%)[17], South Africa (75.6%)[2]; Iceland (42.5%)[18], and USA (0.2%)[19]. Differences in national policies and variations in the usage of antimicrobials, could explain the difference. The USA has not allowed its use[19] while in China, they are commonly used for growth promotion or prophylaxis[17].
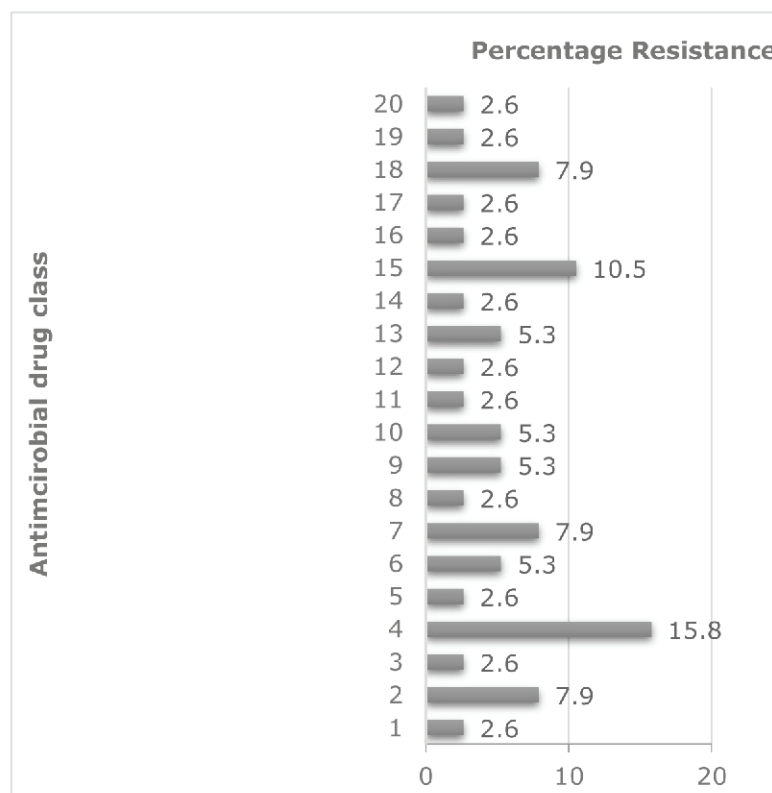
The occurrence of the same resistance phenotype among isolates from the farms may suggest that those isolates are of a single strain and that they have been disseminated in the studied farms. Circulation and dispersion of these resistant bacteria may be attributed to incessant movement and travel of people as well as to movement and trade of animal and food products[20].

Another notable finding is that only contract-growing farms of Company A (28%) showed resistance to 3rd generation cephalosporins. These antimicrobials are used in humans and

resistance observed may indicate use of veterinary cephalosporins ceftiofur, which may result in co-selection. Other countries have withdrawn the use of ceftiofur due to the likelihood that the usage would lead to dissemination of cephalosporin-resistant strains of food-borne bacterial pathogens[21, 22].

The third generation cephalosporins are among the highest priority that CIA used in the treatment of severe Gram-negative infections such as systemic salmonellosis and campylobacteriosis in humans[7]. The observed resistance is of great concern globally, particularly resistance due to extended-spectrum β-lactamases (ESBLs). ESBL-producing strains are often resistant to first-line drugs like fluoroquinolones[23]. The presence of such resistance mechanism may result in fewer options for treatment.

Fig. 2. *E. coli* **resistance profile by antimicrobial drug class.** Abbreviation: SDHFRIC = sulfonamides, dihydrofolate reductase inhibitors and combination

The study revealed that broiler chickens could be an important sources of MDR-*E.coli* bacteria that have the potential to be passed on to humans through the food supply, therefore, pose a public health threat. More research, however, is needed to investigate the impact of AMR and to determine the resistance determinants associated with phenotypic resistance. Genotyping to identify resistance genes has to be carried out.

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