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<Original Article>

Prevalence and antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from ducks in Korea

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Abstract : This study was conducted to investigate the prevalence and antimicrobial resistance of *Salmonella* spp. and *Escherichia* (*E.*) *coli* isolated from ducks in Korea. A total of 400 cecal content samples were collected from 40 duck farms in Korea. Isolated *Salmonella* spp. and *E. coli* strains were 83 and 364 of the 400 cecal samples, respectively. The most prevalent serotype among the 83 *Salmonella* isolates was *Salmonella* Typhimurium (51 isolates: 61.45%). Resistance to the tested antimicrobial agents by *Salmonella* isolates was low except for erythromycin, while the resistance of the *E. coli* isolates to the other tested antimicrobial agents was high and 90.9% (331/364) of *E. coli* isolates showed multi-antimicrobial resistance. Antimicrobial resistance in duck zoonotic pathogens should be of concern to the Korean duck industry, as these pathogens exhibit a high rate of antimicrobial resistance and pose a potential hazard to public health.

Keywords : *Escherichia coli*, *Salmonella* spp., antimicrobial resistance, duck

Introduction

Poultry, and foods originating from poultry, are important sources of food-borne illnesses in humans [4, 23]. Zoonotic bacteria in the digestive tract of poultry may contaminate the final saleable product via the slaughter process, and consumption of contaminated poultry, or the handling of raw poultry, are main causes of human infection [5].

Salmonella spp. (23%) and *Escherichia* (*E.*) *coli* (31%) are the major cause of bacterial food-borne poisoning in Korea [19] and poultry is one of the major reservoirs of human *Salmonella* and *E. coli* infection [6]. Chicken and its meat had the highest *Salmonella* isolation rate among all livestock in Korea [10]. The isolation rates of *Salmonella* spp. from chicken cecal content and meat were 21.3% and 42.3%, respectively [6, 23]. Consumers in Korea have continued to eat increasingly larger quantities of poultry meats; the average annual consumption of the duck meat for person has risen from 0.9 kg in 1995 to 3.40 kg in 2012; therefore, there has been almost a 3.7-fold increase in duck consumption over this 17 years period [13]. These data suggest that the potential for consumer exposure to pathogenic microorganisms in poultry meat has been rising and that the consumption of poultry meats contaminated with *Salmonella* spp. or *E. coli* should be considered as a public health concern.

The duck production industry is an important component of the poultry industry in Korea, second only to the chicken production industry. Approximately 11 million ducks are raised and sold each year from 860 duck farms in Korea [14]; however, while many studies of chicken and chicken meat associated with food-borne pathogens are performed, the research on zoonotic pathogens from ducks has been rarely conducted. Although duck industry has grown rapidly, their antimicrobial resistance studies did not accrue sufficiently.

The emergence of antibiotic resistance in strains of *Salmonella* and *E. coli* is also a serious public health hazard. Antimicrobials have been used for therapy, prevention of disease, and growth and development in livestock production systems. However, the overuse of antimicrobial agents as feed additives could potentially have a negative effect on the susceptibility of microbes to these agents [1], therefore decreasing the overall therapeutic efficacy of antibiotics [1, 22]. Since the problem of antibiotic resistance is directly related to public health, the consideration of its relevance has increased along with social interest in the problem. In addition, overuse of antibiotics in livestock as well as in humans, can lead to the formation of antibiotic resistant bacteria, which have the potential to cause a public health risk to humans and their surrounding environments. Therefore, there is a need to examine the prevalence of antimicrobial resistance in ducks, particularly as these data are currently lacking in the Korean duck industry.

To reduce contamination and infection caused by zoonotic

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pathogens from ducks, the prevalence of zoonotic pathogens and their antimicrobial resistance in ducks must be monitored. In this study, the primary objective was to investigate the isolation rate of *Salmonella* spp. in ducks, and to collect data on their serotypes and antimicrobial resistance profiles. The secondary objective of our work was to examine the antimicrobial resistance of *E. coli* isolated from ducks.

Materials and Methods

Sample collection

Total samples of four hundred ceca were obtained from each forty Korean duck farms in September 2013. We took ten ceca samples per each farm. The entire ceca, which had been removed from the ducks during the slaughter process, were dispatched to a laboratory, where the cecal contents were isolated under aseptic conditions.

Identification of *Salmonella* spp.

Salmonella isolation was performed using a modified version of a previously described method [3, 25]. Samples were incubated in buffered peptone water (BPW; Oxoid, UK) at a ratio of 1 : 10; subsequently, 0.5 mL of the BPW mixture was incubated in 5 mL of tetrathionate broth (Oxoid) at 37°C for 24 h. The tetrathionate mixture was then streaked onto Rambach agar plates (Merck, Germany) and incubated at 37°C. After 24 h, the plates were examined for the presence of assumed *Salmonella* colonies, and the suspected colonies were further streaked onto Rambach agar plates. For confirming the presence of *Salmonella* spp., biochemical identification tests were performed using an API 20E kit (bioMérieux, France).

Salmonella samples were serotyped according to the Kauffmann-White scheme [20]. *Salmonella* colonies were transferred to MacConkey agar (Oxoid) for pure culturing on which they were incubated overnight at 37°C. Samples were plated on the MacConkey agar to react with *Salmonella* O antiserum (Difco, USA), and colonies showing typical agglutination by O antiserum were serotyped with *Salmonella* H antiserum (Difco).

Identification of *E. coli*

E. coli isolation was performed using a modified version of a previously described method [16]. Samples were incubated in BPW at a ratio of 1 : 10, and then 0.5 mL of the BPW mixture was incubated in 5 mL of Tryptone Soya broth (Oxoid) at 37°C for 24 h. The Tryptone Soya mixture was streaked onto MacConkey agar plates and these were incubated at 37°C. After 24 h of incubation, the plates were examined for the presence of assumed *E. coli* colonies, and the suspected colonies were streaked onto MacConkey agar plates. For confirming the presence of *E. coli*, biochemical identification tests were performed using an API 20E kit (bioMérieux).

Table 1. *Salmonella* (*S.*) serotypes isolated from duck in Korea

Serotype	Number of isolates
S. Typhimurium	51
S. Enteritidis	7
S. Hadar	5
S. Indiana	4
S. Give	1
S. Mbandaka	1
Salmonella spp.	14
Total isolates	83

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using a disk diffusion assay, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The following antimicrobial disks (Oxoid) were used for screening of both *Salmonella* spp. and *E. coli*: tetracycline (TE; 30 μg), chloramphenicol (C; 30 μg), ampicillin (AMP; 10 μg), cephalothin (KF; 30 μg), streptomycin (S; 10 μg), gentamicin (CN; 10 μg), sulfamethoxazole/trimethoprim (SXT; 25 μg), nalidixic acid (NA; 30 μg), cefotaxime (30 μg), florfenicol (30 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), erythromycin (E; 15 μg), and kanamycin (K; 30 μg).

Results

Isolation and serotyping of bacteria

Salmonella spp. were isolated from 83 (20.75%) of the 400 duck fecal samples. Within these 83 samples, six serotypes of *Salmonella* spp. were identified: *Salmonella* Typhimurium,

Table 2. Antimicrobial resistance patterns in *Salmonella* spp. isolates

Number of antimicrobials	Antimicrobial resistance pattern	Number of isolates (%)
6	TE-SXT-S-AMP-NA-E	1 (1.2)
4	TE-S-NA-E	1 (1.2)
3	TE-SXT-E	2 (2.4)
	TE-S-E	8 (9.6)
	TE-KF-E	1 (1.2)
	KF-AMP-E	4 (4.8)
	C-NA-E	1 (1.2)
2	S-E	1 (1.2)
	NA-E	8 (9.6)
	AMP-E	2 (2.4)
1	E	54 (65.0)

TE, tetracycline; C, chloramphenicol; AMP, ampicillin; KF, cephalothin; S, streptomycin; CN, gentamicin; SXT, sulfamethoxazole/trimethoprim; NA, nalidixic acid; CTX, cefotaxime; FFC, florfenicol; OFX, oflaxacin; CIP, ciprofloxacin; ENP, enrofloxacin; E, Erythromycin; K, kanamycin.

Table 3. Multi-antimicrobial resistance in *Escherichia* (*E.*) *coli* isolates

Number of antimicrobials	Number of isolates showing antimicrobial resistance (%)
13	2 (0.5)
12	1 (0.3)
11	15 (4.1)
10	7 (1.9)
9	18 (4.9)
8	19 (5.2)
7	43 (11.8)
6	51 (14.0)
5	52 (14.3)
4	33 (9.1)
3	41 (11.3)
2	49 (13.5)
1	33 (9.1)

Table 4. Major antimicrobial resistance patterns in *E. coli* isolates

Antimicrobial resistance pattern	Number of isolates (%)
TE-E	32 (8.8)
E	32 (8.8)
TE-SXT-S-C-AMP-NA-E	19 (5.2)
TE-SXT-S-AMP-NA-E	16 (4.4)
TE-SXT-E	16 (4.4)
TE-SXT-S-E	12 (3.3)
TE-SXT-AMP-NA-E	8 (2.2)
TE-SXT-NA-OF-CI-EN-E	6 (1.6)
TE-SXT-S-C-AMP-E	6 (1.6)
TE-SXT-S-C-F-E	6 (1.6)
TE-SXT-AMP-E	6 (1.6)
TE-AMP-E	6 (1.6)
TE-NA-E	5 (1.4)
NA-E	5 (1.4)
SXT-E	5 (1.4)

Table 5. Antimicrobial resistance in *Salmonella* spp.

	Susceptible (%)	Intermediate (%)	Resistant (%)
TE	83.1	1.2	15.7
SXT	96.4	0	3.6
CN	100	0	0
S	83.1	3.6	13.3
C	98.8	0	1.2
KF	92.8	1.2	6
AMP	89.2	2.4	8.4
NA	85.5	1.2	13.3
CTX	100	0	0
FFC	100	0	0
OFX	100	0	0
CIP	98.8	1.2	0
ENP	91.6	8.4	0
E	0	0	100
K	97.6	2.4	0

Table 6. Antimicrobial resistance in *E. coli*

	Susceptible (%)	Intermediate (%)	Resistant (%)
TE	19.8	1.9	78.3
SXT	37.9	0.5	61.5
CN	94.2	0.5	5.2
S	47.5	6	46.4
C	63.5	4.1	32.4
KF	84.1	12.4	3.6
AMP	49.5	1.9	48.6
NA	51.6	3.3	45.1
CTX	98.1	1.6	0.3
FFC	83.5	0.3	16.2
OFX	79.4	0.5	20.1
CIP	77.2	2.7	20.1
ENP	55.8	20.9	23.4
E	0.3	1.9	97.8
K	72.3	21.2	6.6

Salmonella Enteritidis, *Salmonella* Hadar, *Salmonella* Indiana, *Salmonella* Mbandaka, and *Salmonella* Give. The most frequently isolated serotype was *Salmonella* Typhimurium (51/83 *i.e.*, 61.45%). However, 14 isolates remain unidentified (Table 1). *E. coli* was also isolated from 364 of the 400 samples.

Multi-antimicrobial resistance profiles

Table 2 provides a breakdown of the antimicrobial resistance patterns exhibited by *Salmonella* spp. isolates from the duck ceca. We found that 34.9% of the isolates (29/83) displayed multi-antimicrobial resistance. The most predominant patterns of multi-resistance in *Salmonella* spp. were TE-S-E, in 9.6% of isolates, and NA-E, in 9.6% of isolates.

Tables 3 and 4 provides a muti-antimicrobial resistance and major antimicrobial resistance patterns except for erythromycin exhibited by *E. coli* isolates from the duck ceca. Of the multi-resistance patterns displayed, TE-E resistance was the most common, in 8.8% of isolates, followed by TE-SXT-S-C-AMP-NA-E, shown by 5.2% of isolates.

Antimicrobial susceptibility testing

The results revealed that all *Salmonella* isolates from duck were resistant to the antibiotic erythromycin. Conversely, all isolates were susceptible to gentamicin, cefotaxime, florfenicol, and oflaxacin. The antimicrobial susceptibility test results

from the isolated *Salmonella* spp. are shown in Table 5. Intermediate data were based on a general standard by CLSI.

E. coli isolated from duck were mostly resistant to erythromycin, tetracycline, and sulfamethoxazole/trimethoprim, as shown by 97.8%, 78.3%, and 61.5% of isolates, respectively. The antimicrobial susceptibility test results from the *E. coli* isolates are shown in Table 6.

Discussion

Studies of *Salmonella* spp. isolated from duck have been extensively performed in Southeast Asia, where the duck industry is vast. In these studies, *Salmonella* spp. were isolated from 5.57% (53/950) of duck cecal contents in Vietnam and 39.0% (41/105) in Malaysia [2, 26]. By contrast, not much is known about the zoonotic pathogens in Korea, although the production of duck meats in Korea has increased by almost three times over the past seven years [13]. In a study by Bae *et al*. [4], it was reported that *Salmonella* was isolated from all of the 10 examined duck carcasses, with *Salmonella* Typhimurium being the most predominant serotype. In our study, the isolation rate of *Salmonella* from duck ceca was 20.75%, with *Salmonella* spp. found in 83 of the 400 examined ceca. The *Salmonella* isolates contained 6 different serotypes and *Salmonella* Typhimurium was the most common among those, occurring in 61.45% of the *Salmonella*-positive ceca, followed by *Salmonella* Enteritidis (8.43%). The serotypes of the *Salmonella* isolates from the duck cecal samples showed a similar distribution to those found in chicken; however, the percentage of *Salmonella* Typhimurium in duck was relatively higher than that in chicken [6].

The results of our antimicrobial resistance testing demonstrate that all isolates of *Salmonella* (100%) from duck exhibit erythromycin resistance, whereas 97.8% of the *E. coli* isolates show resistance to this antimicrobial agent. The data for *Salmonella* resistance cohere with a study conducted in Malaysia that all *Salmonella* isolates from duck were resistant to erythromycin [2]. In the studies of chicken, the resistance rates of *Salmonella* isolates to erythromycin have also been found to be relatively high, 64.5% [15].

Resistance rates of *Salmonella* isolates from duck to tetracycline (15.7%), streptomycin (13.3%), ampicillin (8.4%), and nalidixic acid (13.3%) have been found to be considerably lower than those of *Salmonella* isolates from chicken [6, 9, 15, 21]. One explanation for this trend suggests that the amount of antibiotic used in duck production in Korea is lower than that used in chicken production.

Recent studies using molecular techniques indicate that the use of antibiotics in animals farmed for food contributes to the development of antimicrobial-resistant *Salmonella* spp., which can lead to infections in humans [7, 8, 18, 24]. This highlights the need for a continuous management of this practice in order to reduce the occurrence of antimicrobial-resistant *Salmonella* spp. in ducks.

In the present study, the resistance of *E. coli* isolates to antibiotics was also found to be high, with multi-antimicrobial resistance by *E. coli* at serious levels in ducks. The resistance rates of *E. coli* to tetracycline, sulfamethoxazole/trimethoprim, and erythromycin were over 60%, while the resistance rates of *E. coli* to streptomycin, ampicillin, and nalidixic acid exceeded 40%. Moreover, 90.9% of *E. coli* isolates exhibited resistance to more than 2 antibiotics. *E. coli* is a normal component of the flora in gastrointestinal tracts of both humans and animals that is generally non-pathogenic, although it has the potential to cause a broad range of intestinal and extra-intestinal diseases. However, the level of antimicrobial resistance in *E. coli* is considered to be an appropriate indicator of resistance levels in related pathogenic microorganisms [16].

The results of our studies on duck cecal samples indicate that the occurrence of *Salmonella* spp. in these animals is relatively high. The high isolation rate of *Salmonella* spp. at the production level suggests strongly that duck meat for human consumption could be cross-contaminated, which emphasizes the need of developing/improving biosecurity measures against *Salmonella* spp. during meat production.

Furthermore, our data suggest that studies for *Salmonella* spp. isolation from duck meat should be conducted, as they would be expected to reflect the alarming results of previous studies on chicken meat. For example, over the past 10 years, a large amount of researches to identify *Salmonella* spp. in chicken meat have been carried out that have revealed a 22.4–42.3% isolation rate of *Salmonella* according to spot and period samplings [10-12]. In this connection, antimicrobials in chicken were used about 159,290 kg in Korea, 2013 [3]. However, research on *Salmonella* spp. isolated from duck meat appears to be lacking in Korea. These findings suggest that, owing to the increasing consumption of duck meat in Korea, studies of zoonotic pathogens from duck meat, as well as from cecal samples, should be extensively conducted as the most crucial data originating from studies of pathogenic and antimicrobial genes of the zoonotic bacteria from duck. Our findings also suggest that our study has revealed the true extent of antimicrobial resistance in *Salmonella* spp. and *E. coli* from duck. We consider that our data are of a great importance with regard to the establishment of guidelines for antibiotic usage in the duck production industry. These findings indicate that this application of antibiotics should be closely monitored, since increases in antimicrobial resistant bacteria due to the overuse of antibiotics could be potentially harmful to public health.

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