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FULL PAPER

Prevalence and antimicrobial resistance of *Salmonella* isolated from poultry slaughterhouses in Korea

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Abstract

We determined the antimicrobial resistance of *Salmonella* serovars from a total of 154 (44 chilling waters and 110 carcasses) samples collected from 22 poultry slaughterhouses. Standard culture techniques, Kauffmann-White slide agglutination and disc diffusion tests were used to isolate, and identify the serovars and to assess the antimicrobial activity, respectively. A total of 88 isolates belonging to 34 *Salmonella* serovars from 67 (43.5%) positive samples were identified. Among the samples examined, 68.2% (15/22), 22.7% (5/22), and 42.7% (47/110) from the first chilling waters, the last chilling waters, and carcasses were found contaminated with *Salmonella*, respectively. The prevalent serovars were *S.* Enteritidis (12.5%) followed by *S.* Montevideo and *S.* Senftenberg (8.0%). Rare *Salmonella* serovars such as *S.* Aba, *S.* Malmoe, *S.* Westhampton, *S.* Takoradi, and *S.* Baiboukoum in chicken slaughterhouses and *S.* Newbrunswick, *S.* Huddinge *S.* Glostrup, *S.* Dujugu, *S.* Goettingen and *S.* II in duck slaughterhouses were also detected. Among the serovars, 52.3% (46/88) and 21.6% (19/88) were resistant to one antibiotic and more than two antibiotics, respectively. High antimicrobial resistance rates against sulfamethoxazole (39.8%) followed by tetracycline (22.7%), nalidixic acid (21.6%), ampicillin and amoxicillin-clavulanic acid (8.0%), and chloramphenicol (4.5%) were observed. These results suggest more stringent hygienic measures should be taken to reduce the incidence of pathogen contamination in the food chain.

Key Words: antimicrobial resistance, Korea, poultry, prevalence, *Salmonella* serovars

Introduction

Salmonella is one of the most important bacterial pathogens responsible for food poisoning in humans. The organism has been isolated from a range of foods such as meat and dairy products in almost every country in which it has been studied[23]. Several epidemiological reports have also reported poultry products as a primary reservoir of *Salmonella* infection worldwide[23].

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Salmonella is also a major foodborne and waterborne pathogens, with *S*. Enteritidis and *S*. Montevideo more frequently isolated from chicken carcasses and the most common cause of salmonellosis in humans in South Korea[12,7].

The primary method used to characterize members of the genus *Salmonella* is serotyping which is a useful epidemiological tool to detect outbreaks and the emergence of new serotypes, defined by the combination of the surface antigens O, H and Vi. According to the Kaufmann-White scheme, over 2,500 different serotypes have been identified with the majority of these belonging to the *Salmonella enterica* subspecies *enterica*[21].

A variety of *Salmonella* serovars frequently contaminate animals at the farm level facilitating the transfer of pathogens among animals when stress levels are elevated. This has been shown during the transport of animals in which fecal shedding resulted in an increased pathogen load[28]. Consequently, this could result in animals intended for processing to have considerably greater contamination levels and acting as a significant source of cross contamination for other animals and the processing environment. Studies have also shown that chilling waters and the chilling process are a significant source of pathogen contamination in poultry slaughterhouses during chilling[13].

In addition, the emergence of antimicrobial resistant pathogens has become a serious health hazard worldwide. Antimicrobials are used to prevent and treat diseases as well as promote growth in farm animals, exposing a large number of animals to frequently subtherapeutic concentrations[28] and facilitating the development of antimicrobial resistant bacteria that are subsequently transferred to humans through the food chain. Recently, the emergence of antimicrobial resistance in *Salmonella*, has also led to ineffective treatment of salmonellosis[20]. Thus, the objectives of this study were to isolate and estimate the prevalence of *Salmonella* in poultry slaughterhouses in Korea, to identify their serovars and to assess their antimicrobial resistance.

Materials and Methods

Study design and sample collection: Samples were collected from 22 randomly selected poultry slaughterhouses from July to October 2011. All of the slaughterhouses were modern mechanized plants in which Hazard Analysis Critical Control Point (HACCP) and other legal requirements ensuring food safety have been in practice. The first chilling water, followed by the last chilling water and the post chilled carcasses from each slaughterhouse, were sampled. Chilling waters were collected by transfering 50 ml into a sterile specimen cup, and the rinse from the carcasses was collected according to the United States Department of Agriculture, Food Safety and Inspection Service[26] sample collection guideline from the rehang belt prior to the rehanging of the carcasses on the drip line. Each carcass was aseptically placed into a vacuum bag (Cryovag; Sealed Air, USA), and 400 ml of sterile buffered peptone water (BPW; Difco, USA) was added and shaken 50 times. The carcass was replaced on the line, and approximately 50 ml of rinse water was poured into a sterile specimen cup. All the samples were taken and divided into two 225 ml aliquots of BPW.

Bacterial isolation and serotyping: Samples were taken to the laboratory under ambient conditions on the day of collection and incubated at 37°C for 18 h. After pre-enrichment, 0.1 ml of the broth were transferred to 10 ml of Rappaport-Vassiliadis broth (RV broth; Difco, USA), which was prepared according to the manufacturer's instructions, and incubated overnight at 41.5°C. Colonies from the selective enrichment medium were inoculated into Rambach (Difco, USA) agar plates, and incubated at 37°C for 18 hrs. Suspected *Salmonella* colonies on the agar plates were confirmed by identifying the *invA* gene of *Salmonella* with PCR[22]. A total of 145 samples

were examined for the presence of *Salmonella* during the entire study period. Two typical colonies were picked and serotyped according to the scheme of Kauffmann-White, with agglutination and serum neutralization tests[21]. For this purpose, commercial *Salmonella* O and H phase 1 and phase 2 antisera provided by Difco (Becton Dickinson Co., Franklin Lakes, NJ, and USA) were used separately. *Salmonella* strains were sero-grouped by the slide agglutination test using O antiserum and identified by the tube agglutination test using the H antiserum.

Antibiotic susceptibility test: Eighty-eight *Salmonella* isolates were subjected to a susceptibility test against 15 antibiotics on Müller-Hinton agar (Oxoid) with the disc diffusion method. The following antibiotic discs (Difco, Becton Dickinson, USA) were used with the concentration unit as μg per disc: gentamicin (10 μg), kanamycin (30 μg), ampicillin (10 μg), cefotaxime (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), tetracycline (30 μg), sulfamethoxazole-trimethoprim (1.25/23.75 μg), chloramphenicol (30 μg), ceftazidime (30 μg), amoxicillin-clavulanic acid (30 μg), neomycin (30 μg), ceftriaxone (30 μg), ofloxacine (5 μg), and sulfamethoxazole (100 μg). According to the CLSI M100-S21[8] guidelines, inhibition zones were measured and evaluated as susceptible, intermediate, or resistant. An isolate was considered multidrug-resistant if it was resistant to two or more antimicrobials. *Eschershia coli* strain ATCC 25922 was used as a reference strain.

Results

Detection and distribution of Salmonella serovars

A total of 88 *Salmonella* isolates were detected from 43.5% (67/154) of the total samples examined. The level of contamination and their distribution according to the origins of the samples, that is the first chilling waters, the last chilling waters, and chilled carcasses, are summarized in Table 1. All slaughterhouses were found positive with a range of 1–9 serovars per slaughterhouse, and 0–3 serovars per sample were detected from all sample origins. In seven of the slaughterhouses, *Salmonella* contamination was detected only from the post-chilled carcasses. The distribution of the serovars and positive samples in regards to their origin per slaughterhouse is also included in Table 1. From a total of 88 *Salmonella* isolates representing 34 serovars, *S*. Enteritidis was quite frequently isolated (12.5%) followed by *S*. Montevideo and *S*. Senftenberg (8.0%). *Salmonella* Typhimurium and *S*. Hadar (5.7%) were more common at duck slaughterhouses than *S*. Montevideo and *S*. Senftenberg which were more frequent in chicken slaughterhouses. However uncommon *Salmonella* serovars including *S*. Newbrunswick *S*. Huddinge, *S*. Aba, *S*. Malmoe, *S*. Westhampton and others were detected (Table 1).

Antibiotic Susceptibility

From the 88 *Salmonella* isolates tested, 52.3% (46/88) of the isolates were resistant to a single antibiotic and 21.6% (19/88) were resistant to more than one antibiotic while 26.1% (23/88) of the isolates were susceptible to all of the antibiotics tested. A high antibiotic resistance was observed against sulfamethoxazole (39.8%) followed by tetracycline (22.7%), nalidixic acid (21.6%), ampicillin and amoxicillin-clavulanic acid (8.0%), chloramphenicol (4.5%), cefotaxime (3.4%), ceftriaxone and sulfamethoxazole-trimethoprim (2.3%), and ceftazidime and ofloxacin (1.1%). All isolates were susceptible to ciprofloxacin, kanamycin, gentamicin, and neomycin. Resistance from a range of 1–9 antibiotics was observed and the different resistance patterns of *Salmonella* serovars are presented in Table 2.

Discussion

Foodborne diseases are a major public health problem in most countries, despite improvements

Table 1. Distribution of *Salmonella* serovars in poultry slaughterhouses in Korea

Species	Slaughter Houses	First chilling water	Last chilling water	Chilled carcass[a]
Chicken	1	*S*. Montevideo	NC[b]	NC
	2	*S*. Assinie, *S*. Give	NC	*S*. Enteritidis, *S*. Give
	3	*S*. Enteritidis	NC	NC
	4	Serotype I rough:K:1,4	*S*. Enteritidis	*S*. Baiboukoum, Untypable
	5	*S*. Takoradi	*S*. Malmoe	*S*. Malmoe, *S*. Takoradi, Untypable
	6	*S*. Senftenberg	NC	NC
	7	*S*. Newport	NC	*S*. Montevideo, *S*. Typhimurium, *S*. Newport
	8	NC	NC	*S*. Senftenberg, *S*. Aba
	9	NC	NC	*S*. Virchow
	10	*S*. Enteritidis	NC	*S*. Infantis
	11	*S*. Montevideo	*S*. Montevideo	NC
	12	NC	NC	Serotype I Rough:r:1,6
	13	*S*. Kortrijk	*S*. Coquilhativille	NC
	14	*S*. Virchow	NC	NC
	15	NC	NC	*S*. Senftenberg
Duck	16	*S*. Enteritidis, *S*.Typhimurium	NC	NC
	17	*S*. Hadar, Serotype I Rough:L:1,7	NC	*S*. Huddinge, *S*. London, *S*. Typhimurium, *S*. II
	18	*S*. Enteritidis	*S*. Enteritidis, *S*. Typhimurium	*S*. Goettingen, *S*. Hadar, *S*. Newbrunswick, *S*. Enteritidis
	19	*S*. Montevideo	NC	*S*. Mabandaka
	20	NC	NC	*S*. Orion, *S*. Glostrup
	21	NC	NC	*S*. Dujugu, *S*. Westhampton, *S*. Orion *S*. Senftenberg, *S*. Tennessee, *S*. Albert, *S*. Hadar, Serotype I Rough:i:1,6, Untypable
	22	NC	NC	*S*. Typhimurium, *S*. Hadar, *S*. Wippra, Serotype I Rough:i:1,2
Total	22	15/22 (68.2)[c]	5/22 (22.7)	47/110 (42.7)[d]

[a]Five carcasses were taken per slaughterhouse.
[b]No contamination.
[c]No. of positive/No. of slaughterhouse sampled (%).
[d]No. of positive/No. of total carcasses sampled (%).

in hygiene and food processing and educating of food handlers, and consumers[9]. The current study showed an intermediate prevalence of 43.5% for *Salmonella* compared to previous reported trends throughout the world. The overall *Salmonella* prevalence of 43.5% was higher than reports from Nepal (14.5%)[17], Spain (17.9%)[4], South Africa (19.2%)[27], Brazil (2.7%)[19], and Korea (36%)[7] while comparable to a recent report in Korea (42.3%) in retail markets[12]. However, our finding was also lower compared to reports from Portugal (60%)[2] and Thailand (57%)[20]. The following may have contributed to the observed prevalence of *Salmonella* in this study: the initial high pre-slaughter *Salmonella* load, improper handling and the chilling processes during processing. Furthermore, the observed variation in prevalence among studies

Table 2. Antimicrobial resistance patterns of *Salmonella* isolates originated from poultry carcasses and chilling waters

Serovar	Total no. of isolates	Resistance pattern[a]	Origin
S. Malmoe	1	AMC-CTX-RL-TE-AM-NA-SXT-CRO-C	Chicken CW[c]
Serotype I Rough:L:1,7	1	AMC-CTX-RL-TE-AM	Duck CW
S. Enteritidis	2	AMC-NA-RL-C, NA-RL-C	Duck CW, Chicken CW
S. Takoradi	1	AMC-RL-SXT-AM	Chicken CW
Serotype I Rough:r:1,6	1	NA-RL-TE	Chicken carcass
S. II	1	AMC-RL-TE	Duck carcass
S. Newbrunswick	1	RL-TE	Duck carcass
S. London	1	RL-TE	Duck carcass
S. Coquilhativille	1	RL-C	Chicken CW
S. Typhimurium	1	RL-TE	Chicken CW
S. Montevideo	1	NA-RL	Chicken CW
S. Senftenberg	1	NA-RL	Chicken CW
S. Orion	1	AMC-AM	Duck carcass
S. Virchow	1	NA-RL	Chicken carcass
S. Senftenberg	1	NA-CRO	Chicken CW
S. Hadar	1	RL-TE	Duck CW
Untypable	1	AM-RL	Chicken carcass
S. Newport	1	RL-TE	Chicken CW
S. Hadar	4	TE (3)[b], RL (1)	Duck carcass,
S. Wippra	3	TE	Duck carcass
S. Dujugu	1	RL	Duck carcass
S. Enteritidis	5	NA (3)	Chicken CW
		TE (2)	Duck CW
S. Senftenberg	5	AM (1), NA (1)	Chicken CW
		RL (2), OFX (1)	Duck carcass
S. Montevideo	4	NA (3), RL (1)	Chicken CW
S. Typhimurium	2	CTX (1)	Chicken carcass
		RL (1)	Duck CW
Serotype I Rough:K:1,4	1	TE	Chicken CW
Serotype I Rough:i:1,2	2	TE	Duck carcass
S.Takoradi	2	CAZ (1), AMC (1)	Chicken CW
S. Newport	2	NA	Chicken CW
S. Infantis	3	NA (2), RL (1)	Chicken carcass
S. Give	1	AM	Chicken carcass
S. Orion	2	RL	Duck carcass
S. Kortrijk	1	RL	Chicken CW
S. Mabandaka	2	RL	Duck carcass
S. Dujugu	1	RL	Duck carcass
S. Malmoe	3	RL	Chicken carcass
S. Tennessee	1	RL	Duck carcass
Untypable	1	RL	Chicken carcass

[a]AMC, amoxicilline-clavulanic acid; CTX, cefotaxime; RL, sulfamethoxazole; TE, tetracycline; AM, ampicillin; NA, nalidixic acid; SXT, sulfamethoxazole-trimethoprim; CRO, ceftriaxone; C, chloramphenicol; OFX, ofloxacin; CAZ, ceftazidime; CIP, ciprofloxacin.
[b]No. of isolates included.
[c]CW, Chilling water.

may be due to several other factors such as variations in the sampling procedures, sample sizes, and sanitation standards of different slaughterhouses and the test characteristics of different isolation methods used in different settings[14].

Similar to previous reports[3] the effect of chilling to reduce the level of contamination was also revealed in this study by the higher and lower level of contamination from the first chilling water and last chilling water (68.2% and 22.7%, respectively) compared to the carcasses (42.5%). However, the proportion of *Salmonella* positive carcass for ducks did not decrease in view of the fact that, salmonellosis of chickens may result in acute or chronic infections, whereas ducks only experience short subclinical symptoms and typically develop nonlethal chronic infections or carrier status[10]. Additionally, a number of factors have been implicated as contributing to carcass contamination. These have included the feed and water, the individual situations during rearing and at hatcheries, the farm the stress associated with transport and the general contamination that occurs during processing associated with chilling and other handling procedures[28].

All of the 22 organized poultry slaughterhouses were contaminated with at least one serovar of *Salmonella* among which *S.* Enteritidis was the most common. This is in agreement with the report of high prevalence *S.* Enteritidis observed in human salmonellosis, followed by *S.* Senftenberg and *S.* Montevideo throughout the world[15,16]. All of the 35 carcass samples collected from the duck slaughterhouses were contaminated with at least one *Salmonella* serovar. In addition the detection of *S.* Typhimurium from five of the duck slaughterhouses provides further evidence that ducks could be reservoirs of *Salmonella* spp., which is the most commonly identified serovar associated with human infections from ducks in the United States[5]. The result was also in good agreement with the findings of Adzitey *et al.*[1] and MacCrea *et al.*[18] who reported that *S.* Typhimurium was the predominant *Salmonella* serovar isolated from ducks in Malaysia and from the specialty poultry market in California, USA, respectively. In contrast, Tsai *et al.*[25] reported that *S.* Potsdam (31.9%) and *S.* Dusseldorf (18.7%) were the predominant serovars in ducks in Taiwan. Moreover, it is worth mentioning that uncommon *Salmonella* serovars such as *S.* Huddinge, *S.* Aba, *S.* Malmoe, *S.* Westhampton, *S.* Newbrunswick, *S.* Dujugu, *S.* Glostrup, *S.* Baiboukoum, *S.* Assinie, and *S.* Kortrijk rarely reported worldwide were detected indicating the emergence of rare and new *Salmonella* serovars in Korea that needs to be tracked and further investigated.

The isolation of rare *Salmonella* serovars with increasing frequency of antibiotic resistance could be a serious challenge to public health. Our study revealed 21.6% resistance to two or more antibiotics indicating multi-drug resistance in *Salmonella* is quite a problem in Korea because these *Salmonella* serovars originated from poultry meat, the primary source of human infection worldwide. The antibiotic resistance rate shows that a considerable proportion of the isolates were resistant to commonly used antibiotics; particularly, a high percentage of resistance to sulfamethoxazole, tetracycline, and nalidixic acid was observed. This could be due to the wide spread use of antibiotics as prophylaxis, growth promoters and/or treatment in poultry farms. In this study the resistance rate of 21.6% to nalidixic acid could be the result of the frequent use of quinolones in poultry farms, and according to Choi *et al.*[6], Korea is one of the regions with the highest worldwide rates of antibiotic resistance, including a very high frequency of fluoroquinolone resistance for Enterobacteriaceae.

The highest multi resistance pattern was observed in serovar *S.* Malmoe originating from chicken chilling water with resistance to 9 antibiotics including cephalosporins (Table 2). At the same time, other serovars such as *S.*

Typhimurium, Serotype I Rough:L:1,7 and two *S*. Senftenberg isolates had resistance to third generation cephalosporins. These strains demonstrate the dangerous risk of transferring antibiotic resistant bacteria when poultry products are consumed raw because cephalosporins are a lifesaving treatment option in the treatment of invasive salmonellosis in humans[11]. Conversely, the low resistance level to ciprofloxacin was an important finding since ciprofloxacin is clinically essential for the treatment of serious gastrointestinal infections in adults.[24].

The detection of multidrug-resistant *Salmonella* serovars suggests that antibiotic resistance can pose a risk to both humans and animals. Thus, strict guidelines for the use of antibiotics will be necessary to prevent the dissemination and acquisition of antibiotic resistance.

This study confirms the high prevalence of *Salmonella* in the slaughterhouses examined emphasizing the need for more stringent hygienic measures to reduce the incidence of pathogens in the food chain. Furthermore, a wide distribution of serovars isolated along with their high antimicrobial resistance profile circulating in the poultry slaughterhouses of Korea suggests the need for a detailed epidemiological study of *Salmonella* serovars.

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