

## Epidemiological characteristics of *Salmonella* spp. isolated from different stages of commercial swine farms

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**Abstract** : Epidemiological characteristics of a total of 48 swine herd with diarrhea or a history of diarrhea in Gyeongsang-do between 1999 and 2000 were performed to evaluate the prevalence of *Salmonella* spp., their serotypes and antibiotic resistance patterns with respect to the different stages of swine production system. A total of 139 *Salmonella* spp. (21%) were isolated from 662 fecal samples and the overall herd prevalence of *Salmonella* spp. ranged from 12.5% to 88%. The average prevalence of *Salmonella* spp. from swine stages of suckling/nursery, grow/finisher and sow stage were 25.7%, 19.2% and 18.4%, respectively. Ten serotypes of *Salmonella* spp. were identified with a predominance of *S. Typhimurium*, *S. Derby* and *S. Agona*. Twenty-five isolates (18%) were found to be untypable. One hundred and two *Salmonella* isolates (73.4%) resistant to more than 1 antibiotic were characterized by 24 diverse resistance patterns, and their frequency of antibiotic resistance was highest in grow/finisher stage (83.3%). Resistance to tetracycline (TE; 67.6%), sulfamethoxazole (SU; 46.8%) and streptomycin (ST; 28%) was most common and the most common resistance patterns were TE SU (31.4%), TE (21.6%) and TE SU ST (20.6%) in order.

**Key words** : antibiotic resistance, *Salmonella* spp., serotypes, swine

### Introduction

Infection with non-typhoidal salmonellosis continues to be a major public health burden, with 45,000 cases and 400 to 600 deaths reported annually to the Centers for Disease Control and Prevention [7]. In the United States, *Salmonella enterica* serotype Typhimurium and Enteritidis are the two most frequently isolated serovars in human salmonellosis. Along with campylobacteriosis, salmonellosis is considered as one of the two most prevalent foodborne diseases in industrialized countries. In particular, pork meat and pork meat-based products are significant sources of infection in human [3]. Swine can carry *Salmonella* spp. in both the intestinal tract and the mesenteric lymphatic nodes. Stress factors, such as transport to the abattoir, have been shown to promote shedding of *Salmonella* spp.. This release of *Salmonella* spp. contributes to the contamination of carcasses and the environment at slaughterhouse [29].

Of particular concern is the increasing frequency of

multi-drug resistance (MDR) within *Salmonella* strains isolated from zoonotic foodborne infections and dissemination of antibiotic resistance genes among bacterial organisms. One notable MDR serotype Typhimurium definitive phage type 104 (DT104) has been first recognized since its report in many parts of the world [9, 16]. There have been continuing reports for detection and monitoring of multidrug-resistant *S. Typhimurium* and Enteritidis [4, 19]. The ability of bacteria to acquire and disseminate exogenous genes horizontally via mobile genetic elements such as plasmids and transposons has been the major factor in the development of MDR [23, 26]. Recently, gene expression elements called integrons have been described as vehicles for dissemination and acquisition of antimicrobial resistance in many organisms [28]. The antibiotic resistance phenotypes may arise from many different genetic determinants and each determinant may present specific epidemiological features. Despite much research into the characterization of resistance mechanisms among *Salmonella* spp., the prevalence of

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multiple antimicrobial resistance in swine facilities is rarely published. Also, many of the previous reports containing estimates of the prevalence of *Salmonella* spp. have in fact compiled epidemiological data from slaughterhouse or carcasses [8, 14, 25]. The purpose of this study was to evaluate the prevalence of *Salmonella* spp. in swine herd from different stages of production system and to determine the antibiotic resistance of *Salmonella* spp. isolated from the herd.

## Materials and Methods

### Swine herd and sample collection

A total of 48 farrow to finish herd with between 500 and 5,000 pigs per herd in Gyeongsang-do were selected on the basis of diarrhea or a history of diarrhea at the time of this study. A total of 662 fecal samples on the floor of different stages within these farms, such as suckling/nursery, grower/finisher and sow unit, were collected between January 1999 and December 2000. Fecal specimens were randomly sampled from 8 to 16 pigs of each herd. All specimens were taken from freshly defecated feces using sterile cotton swab and were submitted to the laboratory.

### Isolation of *Salmonella* spp.

Isolation of *Salmonella* spp. was performed by conventional methods [13]. Briefly, samples incubated in 10 ml of tetrathionate broth (Difco, USA) at 37°C for 48 h were transferred into 10 ml of Rappaport-Vassiliadis broth (Difco, USA), and incubated at 37°C for another day. Ten  $\mu$ l of the Rappaport-Vassiliadis mixture was streaked onto XLT-4 agar (Difco, USA) plates and the plates were examined for the presence of presumptive *Salmonella* colonies. After the suspected colonies were streaked onto brilliant green agar (Difco, USA) again, the resulting red colonies were tested for the appropriate reactions on triple sugar iron agar (Difco, USA), and were further processed for confirming *Salmonella* spp. by serum agglutination and biochemical identification [11].

### Antimicrobial susceptibility test

After being confirmed as *Salmonella* spp., the isolates were screened for antimicrobial susceptibility by disk-diffusion method [2]. The isolates were tested against amikacin (AK), amoxicillin (AX), ampicillin (AM), ceftiofur (CE), cephalothin (CT), chloramphenicol (CP),

ciprofloxacin (CF), gentamicin (GM), kanamycin (KM), nalidixic acid (NA), streptomycin (ST), sul famethoxazole (SU) and tetracycline (TE).

## Results

A total of 139 *Salmonella* spp. (21%) were isolated from 662 fecal samples, and the overall herd prevalence of *Salmonella* spp. from 48 swine herd ranged from 12.5% to 88%. More than one *Salmonella* spp. was isolated from all farms. Also, the average prevalence of *Salmonella* spp. from swine stages of suckling/nursery, grow/finisher and sow stage were 25.7%, 19.2% and 18.4%, respectively (Table 1). Table 2 shows the prevalence of *Salmonella* serotypes obtained from the farms selected. Various isolates of *Salmonella* spp. were found belonging mostly to serogroup B (66.9%). The most common serotypes identified were Typhimurium, Derby, Agona, Schwarzengrund and Rissen. Twenty-five

**Table 1.** Prevalence of *Salmonella* isolates from different stages in swine herd

Swine stages	No. of fecal samples	No. of isolates	%
Suckling/Nursery	206	53	25.7
Grow/Finisher	250	48	19.2
Sow	206	38	18.4
Total	662	139	21

**Table 2.** Distribution of *Salmonella* serotypes isolated in this study

Serotypes	No. of isolates	%
Typhimurium	34	24.5
Derby	30	21.6
Agona	15	10.8
Schwarzengrund	12	8.6
Rissen	10	7.2
Enteritidis	4	2.9
London	3	2.1
Infantis	3	2.1
Saintpaul	2	1.5
Anatum	1	0.7
Untypable	25	18
Total	139	100

isolates (18%) were found to be untypable.

All *Salmonella* isolates were tested for their resistance to antimicrobial agents of human and veterinary significance. The average prevalence of resistant isolates among farms was 73.4%. The frequency of antibiotic resistance from suckling/nursery, grow/finisher and

**Table 3.** Antibiotic resistance frequency of *Salmonella* isolates from different stages in swine herd

Swine stages	No. of isolates	No. (%) of resistant isolates
Suckling/Nursery	53	42 (79.2)
Grow/Finisher	48	40 (83.3)
Sow	38	20 (52.6)
Total	139	102 (73.4)

**Table 4.** Antibiotic resistance patterns of 102 *Salmonella* isolates

No. of antibiotics	Resistant patterns	No. of isolates	%
8	TE SU ST AM AX CP GM NA	2	1.4
7	TE SU ST AM AX CP GM	1	0.7
6	TE SU ST AM CP NA	1	1.4
	TE SU ST AM AX CP	1	
5	TE SU AM AX CP	1	2.9
	TE SU ST AM CP	2	
	TE SU ST AM GM	1	
4	TE ST AM CT	1	2.2
	AM NA AX CT	2	
3	TE SU ST	21	18.7
	TE AM CT	1	
	TE SU CP	1	
	TE ST AM	1	
	TE ST NA	1	
	SU CP NA	1	
2	TE SU	32	28.1
	TE ST	4	
	TE NA	1	
	SU ST	1	
	ST AM	1	
1	TE	22	18
	ST	1	
	NA	1	
	SM	1	
0	Sensitive to all antibiotics	37	26.6
Total		139	100.0

sow stage were 79.2%, 83.3% and 52.6%, respectively (Table 3). Also, 37 isolates (26.6%) showed no resistance to any of antibiotics tested. One hundred and two isolates resistant to more than 1 antibiotic was characterized by 24 diverse resistance patterns. The most common resistances were shown at tetracycline (67.6%), sulfamethoxazole (46.8%) and streptomycin (28%). Three isolates of total 139 *Salmonella* spp. were resistant to more than 7 antibiotics. The most common resistance patterns were TE SU (31.4%), TE (21.6%) and TE SU ST (20.6%) in order (Table 4). Resistance to chloramphenicol, an antibiotic not used in food animals for more than a decade, was also detected among these isolates (9.8%). All isolates were sensitive to amikacin, ceftiofur, ciprofloxacin and kanamycin.

## Discussion

Point estimates of *Salmonella* spp. prevalence and serotypes could not be considered as reliable indicators of the prevalence of *Salmonella* spp. on farms. This suggested that group level, as opposed to farm or company level events or management practices might be important as potential risk factors for *Salmonella* spp. prevalence in market age pigs [14]. In regard to the prevalence of *Salmonella*, 21% of *Salmonella* spp. were isolated from the intestines, but from only 3% of the enteric lymph nodes in the USA in 1985 [25]. In Korea, Choi *et al.* [8] also reported the 8.1% of *Salmonella* spp. isolation from slaughter pigs. In 1999, *Salmonella* spp. was isolated from 20.7–23.1% of mesenteric lymph nodes and 12.3% of rectal contents of slaughter pigs [17]. However, most of these reports focused the prevalence of *Salmonella* spp. from slaughterhouse or carcasses. Therefore, the prevalence of *Salmonella* spp. in swine herd was evaluated at different stages of production system in this study.

The overall prevalence was estimated at 21% and *Salmonella* spp. were detected in all farms. An USA national estimate of prevalence of *Salmonella* spp. in swine feces showed that the prevalence in pigs and herds were 6.2% and 38.2%, respectively [5]. Choi *et al.* [8] reported the farm prevalence of *Salmonella* spp. to be 1.1% to 4.5% between 1984 and 1985. Also, Kim *et al.* [18] reported that *Salmonella* spp. were isolated from 9.5% of fecal samples and from 7.1% of swine herd. The results in this study were higher compared to these previous reports. This may be explained by the

fact that the selection of herds was not a random representation of all herds, but herds with diarrhea or a history of diarrhea. This study also indicated a wide distribution of this pathogen in the different stages of swine unit with a highest contamination rate in suckling/nursery (25.7%). David *et al.* [10], however, reported high prevalence (18-22%) of *Salmonella* spp. in breeding pigs. Letellier *et al.* [21] also reported the high contamination rate in replacement gilts (15.9%) and finishing unit for gilts (21.9%).

Ten different serotypes were found throughout the swine herd and the most common serotypes of *Salmonella* spp. detected were Typhimurium, Derby and Agona in this study. Kim *et al.* [18] also reported that eighteen serotypes of *Salmonella* spp. were identified in 145 (54.9%) *Salmonella* spp. from swine herd and the most common serotypes of *Salmonella* detected were Typhimurium, Derby, Agona and Rissen in order. In a study on North America swine farms, the most common serotypes detected from feces were Derby, Agona, Worthington and Agona [1]. Fedorca *et al.* [12] reported the serotype Derby, Typhimurium, Agona, Brandenburg and Mbandaka as common serotypes detected in 15 states in USA. Although additional studies with much more samples from as many farms as possible are needed to identify the most common serotypes in domestic swine herd, it seems that several common serotypes are present in different geographic regions.

We found the resistance to tetracycline, sulfamethoxazole and streptomycin to be most common among the isolates tested. It was not unexpected to see the increased resistance to the three antibiotics considering the wide use of those in swine production system for treatment and prevention of disease. Particularly, tetracycline was used as feed additive through the most of pig farms for decades. Moreover, high frequencies of isolates resistant to tetracycline have been detected up to 84.2% in *Salmonella* spp. isolates of swine origin in other countries [15, 24]. Choi *et al.* [8] and Kim *et al.* [18] also reported that tetracycline and streptomycin were the most common resistant antibiotics among *Salmonella* isolates from swine feces. *S.* Typhimurium DT104, a important pathogen known to harbor multiple resistant factor, was commonly found to be penta-resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracyclin. Two isolates (1.96%) of *S.* Typhimurium exhibited the same antibiotic phenotype among the 24 diverse resistance pattern in this study. We found that

77 (55.4%) of the isolates were resistant to two or more of antibiotics tested and that the most common resistance patterns were TE SU (31.4%), TE SU ST (20.6%) and TE (21.6%), a similar result by Fedorca *et al.* [12]. Kim *et al.* [18], however, reported that TE SM (29.7%) resistance was prevalent from a total 145 *Salmonella* isolates from swine feces in Korea in 2000.

On the basis of *Salmonella* spp. prevalence of each farm, antimicrobial resistance and the *Salmonella* spp. prevalence of the farm was not correlated. Isolates from the farm with high prevalence of *Salmonella* spp. showed even less incidence of antibiotic resistance (data not shown). This would support other work that incidence of antibiotic resistance was dependent on age of pig and level of antibiotic use [22]. The fact that lower percentage of resistant isolates in sow compared to that in suckling/nursery and finishing pigs in this study indicated young pigs might be a more likely source of resistant isolates. Callear and Smith [6] suggested that pigs, not the sow, are the primary source of fecal *Escherichia coli* in farrowing barns. Mathew *et al.* [22] also reported young pigs at as early as 7 days age could be a more likely source of the resistance transfer. Other investigators also suggested that resistance might be higher in young animals, because bacteria in their intestinal tract have the increased potential for resistance transfer [20, 27]. Further research on continuous screening and nucleotide sequencing of the antibiotic determinant to identify the gene cassettes as part of spatial and temporal epidemiological investigation of their transmission pattern will be needed. In conclusion, our work identified the wide spread of *Salmonella* spp. and their diverse resistance to commonly used antibiotics within swine herd. Also, we assume that the implementation and improvement of biosecurity procedures on pig farms have a great potential to prevent the maintenance of resistance strains through the contamination cycles.

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