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Serovars distribution and antimicrobial resistance patterns of *Salmonella* spp. isolated from the swine farms and slaughter houses

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Abstract : *Salmonella* spp. is an important pathogen to both public and swine industry. The aim of this study was to investigate the distribution of *Salmonella* serovar and antibiotics susceptibility of the isolates from Korean swine producing systems. A total of 63 (5.28%) *Salmonella* spp. was isolated from 1,194 samples (977 fecal materials and 67 organ samples). The predominant *Salmonella* (*S.*) *enterica* serotype and serovar was group B (69.8%) and *S.* Typhimurium (47.6%), *S.* Derby (20.6%) and *S.* Heidelberg (1.6%). But *S.* Cholerasuis which is characterized host specific by septicemia and enteritis to pigs was not isolated. Antimicrobial susceptibility of the isolates varies as follows: Norfloxacine (75%), Ciprofloxacin (67.5%), Amikacin (60%), Colistin (60%), Enrofloxacin (55%). All of isolates were resistant to Erythromycin, Penicillin, Tetracycline and Lincomycin. The results of this study provided useful information regarding antimicrobial susceptibility and resistance patterns to treat salmonellosis and to prevent emergence of multidrug resistance *Salmonella*.

Keywords : antimicrobial, multidrug, resistance, *Salmonella*, serovar

Introduction

Salmonella spp. is an important pathogen to both public and swine industry [20]. Salmonellosis in swine and poultry is considered as a source of human infection because carcasses can be contaminated through contact with feces, blood, intestine contents of infected animals [4]. The genus of *Salmonella* is a gram negative bacillus and divided over 2,500 different serotypes. Some *Salmonella* serovars can affect multiple host species and it makes a serious problem according to the food chain [21, 22]. *Salmonella* spp. was isolated in the farms, slaughterhouse and food markets and the isolates' serovars were variable.

Recent report revealed a prevalence of *Salmonella* from slaughter house consisting, 13.5% of 2,732 lymph nodes and 4.4% of 1,118 cecal contents, respectively [15]. In another report, *Salmonella* was isolated from 15.72% to 21.25% of pigs in the all groups over the 30 days of age in Korean swine farms, and serovars of 22% were *Salmonella* (*S.*) Typhimurium or *S.* Cholerasuis [14].

Antibiotic administration for prevention and treatment in the infected animals are widely practicing in swine industry. But unfortunately, antibiotic resistances were resulted from the overuse of antibiotics, which is pertaining to serious concern on food safety of the animal product [3]. Currently, many advanced countries which implement good welfare and public health policy, prohibit some antibiotics as growth promoters in animal feedstuff [5, 8]. Because, the frequent use of antibiotics increases the risk of the emergence of multi-drug resistant bacteria from food producing animals and possibly increases chances of these mutants transferring to humans [27]. A number of authors reported that *Salmonella* spp. with multi- or single-antimicrobial resistances was frequently isolated in pork production system [17-19].

The purpose of this study was to investigate the distribution of *Salmonella* serovar and antibiotics susceptibility of the isolates from Korean swine producing systems.

Materials and Methods

Study design and collection of samples

In order to investigate the distribution of *Salmonella*

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serovar, the study was performed using serological and bacteriological methods in the swine farms and slaughter houses. The 62 farms and 2 slaughter houses were randomly selected from a list of swine farms according to their geographical location and herd size. At the selected farms four fecal samples to cross sectional study were collected from each of the compartment which were divided into five groups according to the age of pigs; 4, 8, 12, 18, 25 weeks (± 1 week) and sows. Approximately 10 g of fresh feces which were selected randomly on the floor of each pens were collected into the sterilized tube.

Organ samples including liver, gall bladder, and cecum were obtained during autopsies of pigs showing clinical signs in the farms and from post-evisceration of carcasses in the slaughter houses. Then, all samples were transported to the laboratory for the isolation of *Salmonella* spp. by microbiological culture methods [25].

Bacteriological culture and serotyping

For fecal samples, a sample was inoculated and cultured in 1% buffered peptone water (BPW, 1:10 w/v) at 37°C for 16 h. Afterwards, 100 µL of culture fluid were transferred into the Rappaport and Vassiliadis broth (RV, 1:100 v/v, MERK) and incubated at 42°C for 24 h, and this procedure was repeated for all fecal samples. Colonies from selective enrichment medium were streaked on *Salmonella shigella* (SS) agar, xylose lysine deoxycholate (XLD) agar, Rambach agar and MacConkey agar plates, and incubated at 37°C for 18 h. The presence of *Salmonella* isolates were confirmed by biochemical tests using BBL crystal ID system (BD, USA) and serological tests. The organ samples except gall bladder sac were homogenized and then inoculated by the same procedure described above.

All isolates were serotyped by agglutination test according to the Kauffmann-White scheme using *Salmonella* polyvalent somatic (O) and flagella (H) antiserum (DIFCO, USA) [9].

Antimicrobial susceptibility

Antibiotics susceptibility of the *Salmonella* isolates was tested by the agar diffusion method, using 20 kinds of Sensidisk (BBL, USA) according to the method described by National Committee for Clinical Laboratory Standards.

Antibiotics disks used for the test were: amikacin (An), ampicillin (Am), amoxicillin-clavulanic acid (Amc), carbenicillin (Cb), cefazolin (Cz), cephalothin (Cf), ciprofloxacin (Cip), chloramphenicol (C), colistin (Cl), erythromycin (E), enrofloxacin (Enr), gentamicin (Gm), kanamycin (K), lincomycin (L), neomycin (N), norfloxacin (Nor), oxytetracycline (T), penicillin (P), streptomycin (S) and trimethoprime (Tmp). The reference bacteria such as *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used for quality control of the test.

***Salmonella* Cholerasuis-specific polymerase chain reaction (PCR)**

Salmonella Cholerasuis genes were amplified by PCR following the protocol of a previous study [6]. Briefly, 1 mL of the final bacteriological culture broths was centrifuged to harvest enriched bacteria at 8,000 rpm for 5 min. The harvested cells were lysed by boiling at 100°C for 10 min and then centrifugated at 4,000 rpm for 30 sec.

Five microliters of the supernatant were mixed with the PCR premix (iNtRON Biotechnology, Korea; included i-Taq DNA polymerase 2.5 U, dNTPs 2.5 mM), each 10 pmol of sense and antisense primers, and added diethyl pyrocarbonate (DEPC) treated water to 20 µL. *S. cholerasuis* ATCC 13312 and distilled water were used as positive and negative control. PCR conditions were initial denaturation at 94°C for 10 min, and then 35 cycle of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and final extension at 72°C for 7 min. Expect size of amplicons is 963 bp in length.

Results

A total of 977 fecal materials and 67 organ samples was collected and analyzed for *Salmonella* infection in pigs. *Salmonella* spp. was recovered from 63 of 1,194 samples (5.28%). More specifically, *Salmonella* spp. was isolated from 1.5% of fecal sample from the cross section study, 19.4% of organ samples and 19.3% of fecal samples at holding area in the slaughter house (Table 1).

The predominant *Salmonella enterica* serotype was group B (69.8%) which comprised of *S.* Typhimurium (47.6%), *S.* Derby (20.6%) and *S.* Heidelberg (1.6%), followed by group C *S.* Rissen (17.5%) and group E1 *S.* London. The most frequent isolates serovar was *S.* Typhimurium, but *S.* Cholerasuis (group C) was neither isolated in the bacterial culture nor detected by PCR (Table 2).

Table 1. Isolation frequency of *Salmonella* spp.

	Cross section study	Organ sample	Slaughter house	Total
No. of samples (No. of farms)	941 (48)	103 (22)	150 (15)	1,194 (85)
No. of isolated salmonella (No. of Farm)	14 (6)	20 (5)	29 (10)	63 (21)
Recovery rate % (Farm level %)	1.5 (12.5)	19.4 (22.7)	19.3 (66.7)	5.28 (24.7)

Table 2. Distribution of *Salmonella* (*S.*) serovar by serum agglutination test

O group		No. of bacteria	Isolate rate (%)
B	*S.* Typhimurium	30	47.6
	S. Derby	13	20.6
	S. Heidelberg	1	1.6
C	*S.* Rissen	11	17.5
E1	*S.* London	1	1.6
Other	–*	7	11.1
Total		63	100

*The isolates which un-defined serovar are followed as serogroup B (5 isolates), D1 (1 isolate) and unknown serogroup (1 isolate).

As for the results of antibiotics susceptibility test 75% of the isolates were susceptible to Nor. Antimicrobial susceptibility of the isolates varies as follows; Cip (67.5%), An (60%), Cl (60%), Enr (55%), G (55%), respectively. The isolates were highly susceptible to Quinolone antibiotics such as Cip and Nor, except Enr. On the other hand the isolated *Salmonella* strains showed resistance to a few antibiotics such as E, P, T, L (Table 3).

The result of antimicrobial resistance pattern analysis in 40 isolates revealed various and different resistance combinations of 3 to 15 antimicrobials. The three frequent antimicrobial resistance patterns were CfELTP (22.5%), AmAmcCbCfCEGmKLNTPSTmp (7.5%) and AmAmc CbCzCfCipCEEnrLTPSTmp (7.5%), respectively. The

Table 3. Antimicrobial susceptibility of *Salmonella* spp. isolated from pigs

Antibiotics	n	Prevalence			Susceptibility (%)
		Resistance	Intermediate	Susceptibility	
Amikacin (An)	40	5	11	24	60.0
Ampicillin (Am)	40	22	4	14	35.0
Amoxicillin-clavulanic acid (Amc)	40	22	3	15	37.5
carbenicillin (Cb)	40	26	14	0	0.0
cefazolin (Cz)	40	17	7	16	40.0
Cephalothin (Cf)	40	36	3	1	2.5
Ciprofloxacin (Cip)	40	4	9	27	67.5
Chloramphenicol (C)	40	16	5	19	47.5
Colistin (Cl)	40	1	15	24	60.0
Erythromycin (E)	40	40	0	0	0.0
Enrofloxacin (Enr)	40	14	4	22	55.0
Gentamicin (Gm)	40	16	2	22	55.0
Kanamycin (K)	40	11	16	13	32.5
Lincomycin (L)	40	40	0	0	0.0
Neomycin (N)	40	11	23	6	15.0
Norfloxacin (Nor)	40	0	10	30	75.0
Oxytetracycline (T)	40	39	0	1	2.5
Penicillin (P)	40	40	-	0	0.0
Streptomycin (S)	40	28	9	3	7.5
Trimethoprime (Tmp)	40	19	5	16	40.0

An: 30 μg, An: 10 μg, Amc: 30 μg, Cb: 100 μg, Cz: 30 μg, Cf: 30 μg, Cip: 5 μg, C: 30 μg, Cl: 10 μg, E: 15 μg, Enr: 5 μg, Gm: 10 μg, K: 30 μg, L: 2 μg, N: 30 μg, Nor: 10 μg, T: 30 μg, P: 10 μg, S: 10 μg, Tmp: 5 μg.

Table 4. Antimicrobial resistance patterns of the *Salmonella* spp.

No. of antibiotics (resistance %)	Resistance pattern	Number of isolate
3 (2.5%)	ELP	1
4 (2.5%)	ELTP	1
5 (22.5%)	CfELTP	9
6 (2.5%)	EGmLTPS	1
7 (5.0%)	CfCELTPS	1
	CbCfEKLPS	1
8 (2.5%)	CbCELTPSTmp	1
9 (5.0%)	AmAmcCbCfELTPTmp	1
	AnCfClELNTPS	1
10 (5.0%)	AmAmcCbCfELTPSTmp	1
	CbCfCipCEEnrLTPS	1
11 (5.0%)	AmAmcCbCzCfEGmLTPS	2
12 (2.5%)	AnAmAmcCbCfELNTPSTmp	1
13 (10.0%)	AmAmcCbCzCfCEEnrGmLTPS	2
	AmAmcCbCzCfCEEnrLTPSTmp	2
14 (17.5%)	AmAmcCbCfCEGmKLNTPSTmp	3
	AmAmcCbCzCfCipCEEnrLTPSTmp	3
	AnAmAmcCbCzCfEEnrGmLTPSTmp	1
15 (17.5%)	AmAmcCbCzCfCEEnrGmKLTPSTmp	2
	AmAmcCbCzCfCEGmKLNTPSTmp	1
	AmAmcCbCzCfEEnrGmKLNTPSTmp	1
	AmCbCzCfCEEnrGmKLNTPSTmp	1
	AnAmAmcCbCzCfCEEnrGmKLTPS	1
	AnAmcCbCzCfCEGmKLNTPSTmp	1
Total (100%)		40

The underline indicate AmAmcCbCzCfP resistance pattern. The shadow indicate CfELTP resistance pattern.

isolates which contain beta-lactam antimicrobials Resistance pattern (AmAmcCbCzCfP) was found in 15 isolates (37.5%) (Table 4).

Discussion

In this study, *Salmonella* was isolated from fresh feces, cecal contents and organs including lung, liver, gall bladder and cecum. The average of recovery rate was 5.28% (63/1,194) of total samples which consist of 1.5% (14/941) of fecal samples from the cross section study in the farm level, 19.4% (20/103) of organ samples and 19.3% (29/150) of fecal samples in slaughter houses. The prevalence of *Salmonella* spp. at farm level was 12.5% (6/48) for cross section study, 22.7% (5/22) for organ samples and 66.7% (10/15) for pigs at slaughter houses. Prevalence of *Salmonella* from organs (autopsied in the farm) and at slaughter houses was higher than that in the cross section study. The differences in recovery rates in the present study indicate that the shedding of *Salmonella* augments by the poor health condition of pigs; *Salmonella* infected pigs could act as subclinical carrier when influenced by stress factors such as noise, mixing with other groups, high stock density, change of environment, which subsequently increase the number of excreted *Salmonella* spp. present in the feces [26].

The serovars of 56 isolates were confirmed as *S.* Typhimurium, *S.* Derby, *S.* Rissen, *S.* Heidelberg and *S.* London by standard typing method. And the other 7 isolates were defined partially that just defined the type of somatic antigens (O). Among the isolates, the serogroup B was the most frequent (69.8%) and followed by the serogroup C (17.5%, *S.* Rissen). There are two similar papers about the sero-prevalence of *Salmonella* in Korea. Lee *et al.* [15] and Kim *et al.* [14] have reported that the most frequently isolated salmonella was serogroup B which showed 69.5% and 69.3%, respectively. Some *Salmonella* serovars are cause a serious disease depending to host animal. The results in present study showed higher prevalence rates than those in these previous

reports. *S. enterica* serovar group B and C1 are commonly isolated in swine farm and slaughter house. Group D1 is frequently isolated in poultry industry.

In this study, the most commonly isolated serotype was *S.* Typhimurium (33.3%). This serovar is very important to the public health, because it is a zoonotic bacterium and frequently isolated from the swine production system. Similar results were reported in the prevalence surveys for *Salmonella* at the swine farm or slaughter house in Japan [10, 13], Italy [16], Spain [11], Ireland [17, 23], Denmark [2] and the USA [12]. These similar results which show that *S.* Typhimurium is widely distributed over the world emphasize, the needs for a standard management protocol to avoid introduction and transmission of *Salmonella* in swine herds and slaughter houses. When the salmonella infected pigs are transported into slaughter house, they could excrete the pathogen at the lairage, contaminate carcasses during the slaughtering process and residues with spray dust in the space of slaughterhouses [1, 28]. *S.* Cholerasuis is condemned as the serovar which was commonly isolated in the swine farms [7]. But in this study, *S.* Cholerasuis was not determined in isolation by culture and detection of specific gene (*flic*) by PCR (the data was not shown).

The present study demonstrated that the antimicrobial resistance and emergence of multidrug resistance were seriously higher than in the past years or in other countries [12, 14, 15]. Lee *et al.* [15] reported that 315 *Salmonella* isolates from Dec. 2000 to Nov. 2001 in Korean slaughter houses were not resistant (< 1%) to Gm, Amc, Cz, Cf, Cl, Cip and Nor. In contrast, Kim *et al.* [14] reported that 114 *Salmonella* spp. isolated in 2005 in Korean swine farms were resistant to T (100%), P (100%), Am (92.98%), Sulfametoxazol/Tmp (89.47%) and L/S (81.58%). Rayamajhi *et al.* [24] showed the resistance to S (94.1%), T (90.1%), Am (64.7%), C (56.8%) and Gm (54.9%). In comparison to the finding of the previous studies showed that *Salmonella*'s resistance tends to increase and become more complex.

The antimicrobial resistance patterns in the present study could be classified to the 24 patterns which consist of antimicrobials combinations from 3 to 15 different kinds of drugs. The major antimicrobial resistance pattern was CfELTP (22.5%). The CfELTP including resistance pattern was 85% in 40 isolates. The other pattern combination of frequent resistant antimicrobial was the pattern including AmAmcCbCfELTPSTmp (37.5%). In the similar study, Huang *et al.* [12] showed that the resistance pattern in 197 *Salmonella* isolates from 2003 to 2005, 27.4% of isolates were not resistant or resistant to one antimicrobial (Florfenicol, Spectinomycin, Tetracycline) among 13 antimicrobials. García-Feliz *et al.* [11] reported that less than 10% of the isolates were resistant to Amc, N, Cf, Apramycin and Gm, and multi-resistance(resistant to four or more drugs) was detected from more than 50% of the isolates in Spain from Mar. 2003 to Feb. 2004. But in this study, the minimum resistance pattern was three antimicrobials resistance (ELP).

Conclusion

The results of this study provided useful information regarding antimicrobial susceptibility and resistance patterns to treat salmonellosis and to prevent emergence of multidrug resistance bacteria.

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