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Full Length Research Paper

Prevalence and antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from pigs at slaughterhouses in Korea

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The objective of this study was to ascertain the nationwide prevalence and antimicrobial resistance rates of *Salmonella* spp. and *Escherichia coli* amongst domesticated finisher pigs. Fecal samples (n=840) were collected at 84 slaughterhouses in Korea in May 2009. *Salmonella* spp. was isolated from 21 of the 840 samples (2.5%), and comprised the following isolated serotypes: *Salmonella rissen*, *Salmonella derby*, *Salmonella typhimurium*. Antimicrobial susceptibility testing was performed for eight antimicrobials. *Salmonella* resistance was tetracycline (76.19%); nitrofurantoin (38.10%); kanamycin (33.33%); chloramphenicol, sulfamethoxazole/trimethoprim and cephalothin (28.57%); polymyxin B (9.52%); and ampicillin/sulbactam (4.76%), and *E. coli* resistance was tetracycline (87.11%); chloramphenicol (66.24%); kanamycin (51.68%); sulfamethoxazole/trimethoprim (51.29%); cephalothin (8.38%); nitrofurantoin (5.15%); ampicillin/sulbactam (4.64%); and polymyxin B (0.52%). Tetracycline resistance was most common. Surprisingly, 28.57 and 66.24% of the *Salmonella* spp. and *E. coli* isolates, respectively, were resistant to chloramphenicol, which has been banned from agricultural use in Korea for some time. A wide range of strains displayed multi-antimicrobial resistance: 14 out of 21 (66.66%) and 611 out of 776 (78.72%) of the *Salmonella* and *E. coli* isolates, respectively. *Salmonella* spp. and *E. coli* demonstrate an appreciable broad-spectrum, (multi)-antimicrobial resistance, which is a potential public health concern. A continuous antibiotic surveillance program may be worthwhile.

Key words: Swine, pig, *Salmonella* spp., *Escherichia coli,* antimicrobial resistance, slaughterhouse.

INTRODUCTION

Pork products have long been a popular dinner item, which has increased the potential exposure of consumers to food contaminated by pathogenic microorganisms. Demand for safe pork products has been increasing, as the demand for livestock product safety, in terms of agricultural antibiotic usage, is increasing.

Pigs are a major reservoir of bacterial zoonotic pathogens such as *Salmonella* (McDowell et al., 2007; Käsbohrer et al., 2000; Mainar-Jaime et al., 2008; Lomonaco et al., 2009), Campylobacter (Padungtod et al., 2008; Denis et al., 2009) and *Escherichia coli* (Teshager et al., 2000). Except for self-consumption and scientific research, finisher pigs in Korea are slaughtered at slaughterhouses (Processing of Livestock Products Act). According to this law, the slaughterhouses in every city become the destination of the finisher pigs from the surrounding region. In the previous study, some reports evaluated prevalence of *Salmonella* spp. in Korea. But, the sampling area in these studies was confined to a few provinces or only involved farms (Suh et al., 2005; Choi et al., 1986; Kim et al., 2007). In this study, the infection rates of finisher pigs could be estimated without regional disproportion by investigating *Salmonella* prevalance from pig feces in every slaughterhouse in Korea.

Salmonella is a prominent food borne disease-causing bacterium and zoonotic pathogen. Pigs can be infected by a variety of serotypes of *Salmonella*. Some infected pigs display clinical symptoms of salmonellosis, while others are asymptomatic. However, even the latter can

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contaminate pork products (Griffith et al., 2006). In Europe, it has been estimated that 10 to 23% of all human salmonellosis are due to consumption of contaminated pork and its products (Mainar-Jaime et al., 2008). So, many countries recognized pigs as being a principal source of salmonellosis (McDowell et al., 2007; Käsbohrer et al., 2000; Mainar-Jaime et al., 2008). There is a significant correlation between the number of pigs harboring *Salmonella* in their feces while alive and the number of *Salmonella*-infected carcasses (Berends et al., 1997). Isolating *Salmonella* from the feces of pigs before carcass inspection can be expected to prescreen for *Salmonella* infection.

E. coli is a normal inhabitant of the gastrointestinal tract and strains causing a broad variety of intestinal and extraintestinal disease in swine have been described (Fairbrother et al., 2006). *E. coli* in the intestinal tract of pigs is generally non-pathogenic (Choi et al., 2002). But, the level of resistance in *E. coli* is considered to be appropriate indicator for resistance to various antimicrobials in related pathogenic microorganisms (Lim et al., 2007).

In this study, fecal samples were collected from all 84 authorized slaughterhouses in Korea. The primary objective was to estimate the prevalence of *Salmonella* spp. in pigs and to collect data on serotype and antimicrobial resistance. A secondary objective was to assess the antimicrobial resistance of *E. coli*.

MATERIALS AND METHODS

Sampling method

Fecal samples from pigs were collected in May 2009 by meat inspection staff at the 84 slaughterhouses and sent for laboratory analyses. Ten fresh fecal specimens were randomly sampled from lairage of each slaughterhouse using transport medium swab (Copan, Brescia, Italy).

Isolation and confirmation of *Salmonella*

Salmonella isolation was performed using a modification of previously described method (Käsbohrer et al., 2000; Mainar- Jaime et al., 2008). Samples were incubated in buffered peptone water (BPW; Oxoid, Hampshire, England) at a ratio of 1:10. The 0.5 ml BPW mixture was incubated in 5 ml of tetrathionate broth (Oxoid, Hampshire, England) at 37°C for 24 h. A tetrathionate mixture was streaked onto XLD agar (Oxoid, Hampshire, England) plates and incubated at 37°C. After 24 h, the plates were examined for the presence of presumptive *Salmonella* colonies, and suspected colonies were streaked onto XLD agar (Oxoid) plates. For confirming *Salmonella* spp, biochemical identification tests were performed using an API 20E kit (bioMérieux, Marcy l'Etoile, France).

Salmonella serotyping

Salmonella sample was serotyped according to the Kauffmann-White scheme (Popoff et al., 1993). *Salmonella* colonies were transferred to MacConkey agar (Oxoid, Hampshire, England) for pure culturing and incubated overnight at 37°C. Samples on the MacConkey agar reacted with *Salmonella* O antiserum (Difco, USA). Colonies showing typical agglutination by O antiserum were serotyped with *Salmonella* H antiserum (Difco, USA).

Isolation and confirmation of *E. coli*

E. coli isolation was performed using a modification of previously described method (Lim et al., 2007). Samples were incubated in buffered peptone water (BPW; Oxoid, Hampshire, England) at a ratio of 1:10. The 0.5 ml BPW mixture was incubated in 5 ml of Tryptone Soya broth (Oxoid, Hampshire, England) at 37°C for 24 h. The Tryptone Soya mixture was streaked onto MacConkey agar (Oxoid, Hampshire, England) plates and incubated at 37°C. After 24 h, the plates were examined for the presence of presumptive *E. coli* colonies, and suspected colonies were streaked onto MacConkey agar (Oxoid) plates. For confirming *E. coli* , biochemical identification tests were performed using an API 20E kit (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using a disk diffusion assay following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009), using the following antimicrobial-containing discs (Oxoid , Hampshire, England) for *Salmonella* and *E. coli*: tetracycline (TE, 30 g), polymyxin B (PB 300 unit), chloramphenicol (C, 30 g), cephalothin (KF, 30 g) kanamycin (K, 30 g) sulfamethoxazole/trimethoprim (SXT, 25 g), ampicillin/ sulbactam (SAM, 20 g), and nitrofurantoin (F, 300 g).

RESULTS

Salmonella isolation

A total of 21 *Salmonella* spp. (2.5%) were isolated from the 840 fecal samples. The prevalence of *Salmonella* isolates by district is summarized in Table 1.

Salmonella serotyping

Three serotypes of *Salmonella* spp. were identified: *Salmonella rissen*, *Salmonella derby*, and *Salmonella typhimurium*. The most frequently isolated serotype was *S. rissen* (52.38%: 11/21) . *S. derby* and *S. typhimurium* accounted for 14.28 and 9.52%, respectively, with five isolates not being identified.

Antimicrobial susceptibility test in *Salmonella* spp.

Antimicrobial susceptibility test results conducted in the *Salmonella* spp. appear in Figure 1. The highest resistance (R: 76.19%) was to TE. Table 2 summarizes multi-resistance (more than two antibiotics) results in *Salmonella* spp. Of the isolates, 66.66% (14/21) displayed multi- resistance. Most predominant patterns of multi-resistance in *Salmonella* were TE-SXT-K-F (9.52%)

Table 1. Prevalence of *Salmonella* isolates by district in Korea.

Districts	No. of samples	No. of isolates
Seoul	10	-
Busan	10	-
Daegu	10	-
Incheon	10	-
Gwangju	20	-
Daejeon	10	-
Ulsan	20	-
Jeju	10	1
Gyeonggi	120	6
Gangwon	90	1
Chungbuk	100	2
Chungnam	70	1
Jeonbuk	100	1
Jeonnam	80	1
Gyeongbuk	90	-
Gyeongnam	90	5
Total	840	21 (2.5%)

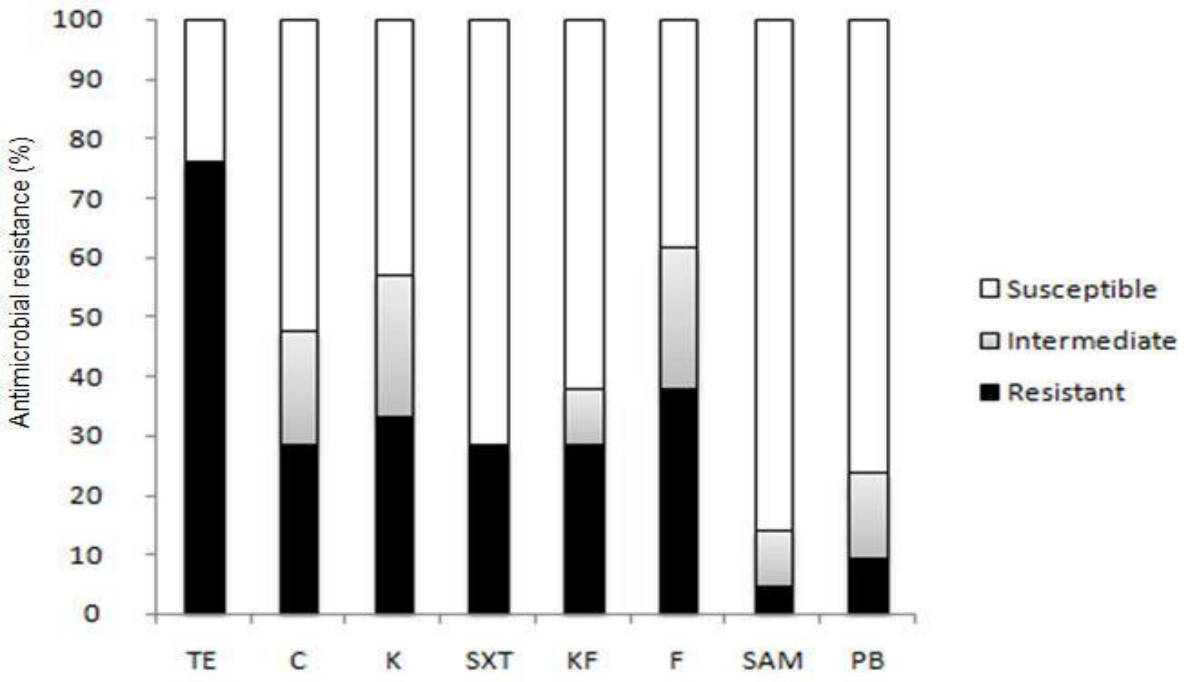

Figure 1. Antimicrobial resistance in *Salmonella* spp. TE: tetracycline, C: chloramphenicol, K: kanamycin, SXT: sulfamethoxazole/trimethoprim, KF: cephalothin, F: nitrofurantoin, SAM: ampicillin/sulbactam, PB: polymyxin B.

and TE-F (9.52%).

Antimicrobial susceptibility test in *E. coli*

E. coli was isolated from 776 of the 840 samples. Figure 2 shows the antimicrobial resistance results. Resistance was most prevalent to TE (87.11%) and C (66.24%). SXT and K had similar rates of resistance and susceptibility. Table 3 summarizes the resistance patterns and the prevalence of multi-resistance. Of the *E. coli* isolates from pig feces, 78.72% showed resistance to more than two

Table 2. Number of resistant antimicrobials and antimicrobial resistance patterns in *Salmonella* spp. (n=21).

No. of resistant antimicrobials	No. of isolates (%)	Antimicrobial pattern *	No. of isolates (%)
5	2 (9.52)	TE-SXT-K-C-KF	1 (4.76)
		TE-SXT-C-SAM-KF	1 (4.76)
4	6 (28.56)	TE-SXT-K-F	2 (9.52)
		TE-C-F-PB	1 (4.76)
		TE-SXT-C-KF	1 (4.76)
		TE-C-F-PB	1 (4.76)
		TE-SXT-K-C	1 (4.76)
3	1 (4.76)	TE-K-F	1 (4.76)
2	5 (23.8)	TE-K	1 (4.76)
		KF-F	1 (4.76)
		TE-KF	1 (4.76)
		TE-F	2 (9.52)
1	5 (23.8)	TE	3 (14.29)
		K	1 (4.76)
		KF	1(4.76)
0	2(9.52)		2(9.52)

*:TE-tetracycline, C-chloramphenicol, K-kanamycin, SXT-sulfamethoxazole/trimethoprim, KF-cephalothin, F-nitrofurantoin, SAM-ampicillin/sulbactam, PB-polymyxin B

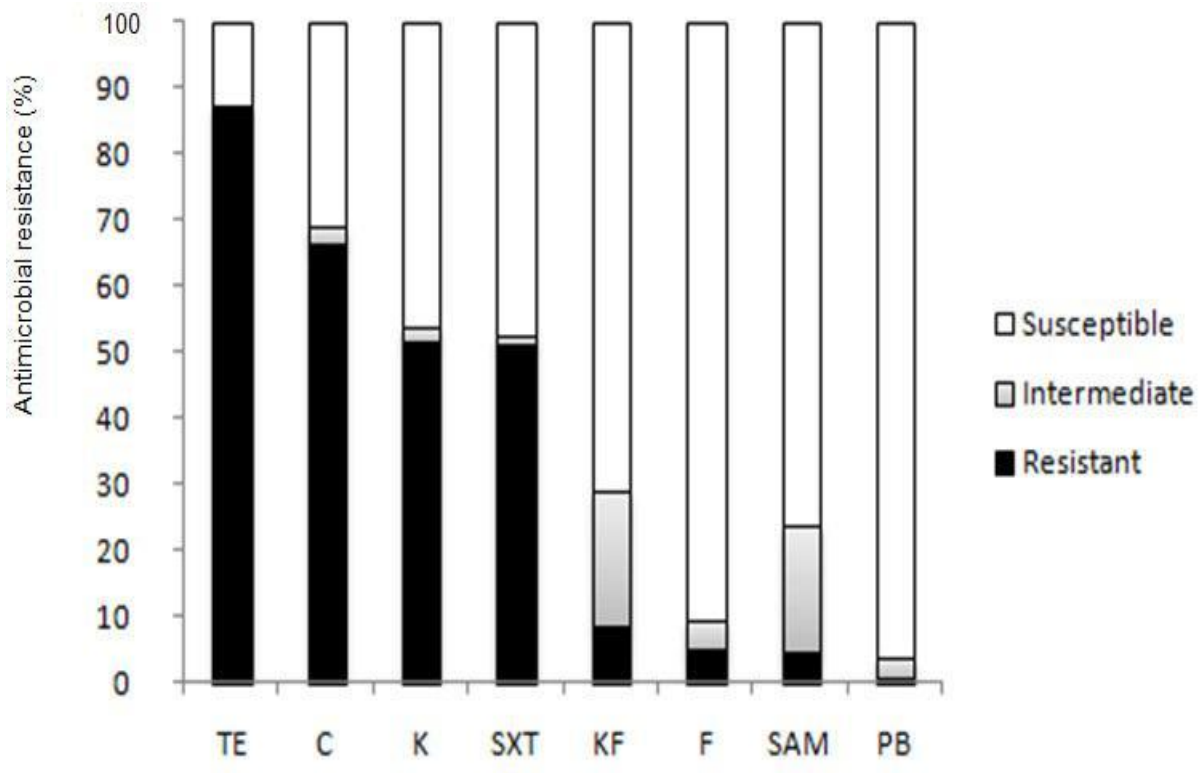

Figure 2. Antimicrobial resistance in *E. coli*. TE: tetracycline, C: chloramphenicol, K: kanamycin, SXT: sulfamethoxazole/trimethoprim, KF: cephalothin, F: nitrofurantoin, SAM: ampicillin/sulbactam, PB: polymyxin B.

Table 3. Number of resistant antimicrobials and antimicrobial resistance patterns in *E. coli* (n=776).

No. of resistant antimicrobials	No. of isolates (%)	Antimicrobial pattern*	No. of isolates (%)
7	5 (0.64)	TE-SXT-K-C-SAM-KF-F	5 (0.64)
6	15 (1.93)	TE-SXT-K-C-F-PB	3 (0.39)
		TE-SXT-K-C-SAM-KF	5 (0.64)
		TE-SXT-K-C-SAM-F	1 (0.13)
		TE-SXT-K-C-KF-F	6 (0.77)
5	48 (6.18)	TE-SXT-C-F-PB	1 (0.13)
		TE-SXT-K-C-SAM	13 (1.68)
		TE-SXT-K-C-KF	14 (1.80)
		TE-SXT-C-SAM-KF	2 (0.26)
		TE-K-C-SAM-KF	1 (0.13)
		TE-SXT-K-C-F	17 (2.19)
4	203 (26.15)	TE-SXT-K-C	177 (22.81)
		TE-SXT-C-SAM	1 (0.13)
		TE-SXT-C-KF	8 (1.03)
		TE-SXT-C-F	3 (0.39)
		TE-K-C-SAM	4 (0.52)
		TE-K-C-KF	6 (0.77)
		TE-K-C-F	1 (0.13)
		SXT-K-C-KF	1 (0.13)
		TE-C-SAM-KF	1 (0.13)
		TE-K-KF-F	1 (0.13)
3	193 (24.89)	TE-SXT-K	25 (3.22)
		TE-SXT-C	87 (11.21)
		TE-SXT-KF	2 (0.26)
		TE-K-C	64 (8.25)
		TE-K-SAM	1 (0.13)
		TE-K-KF	2 (0.26)
		TE-C-SAM	2 (0.26)
		TE-C-KF	9 (1.16)
		SXT-K-C	1 (0.13)
2	147 (18.94)	TE-KF	2 (0.26)
		TE-SXT	17 (2.19)
		TE-K	49 (6.31)
		TE-C	72 (9.28)
		SXT-K	1 (0.13)
		SXT-C	6 (0.77)
1	84 (10.82)	SXT	2 (0.26)
		K	3 (0.39)
		C	3 (0.39)
		F	2 (0.26)
		TE	74 (9.54)
0	81 (10.43)		81 (10.44)

*:TE-tetracycline, C-chloramphenicol, K-kanamycin, SXT-sulfamethoxazole/trimethoprim, KF-cephalothin, F-nitrofurantoin, SAM-ampicillin/sulbactam, PB-polymyxin B.

antibiotics. In the multi-resistance pattern, TE-SXT-K-C resistance was most common (177/776, 22.81%) followed by TE-SXT-C (87/776, 11.21%).

DISCUSSION

Salmonella is considered the most important bacterial pathogen that causes food poisoning in pork. The entry of *Salmonella* carrier pigs into slaughterhouses is a major source of meat contamination and of introduction of *Salmonella* into the food chain (McDowell et al., 2007; Käsbohrer et al., 2000; Berends et al., 1997; Padungtod et al., 2008; Padungtod et al., 2006) . For this reason, several studies have investigated the *Salmonella* prevalence from the feces of pigs in slaughterhouses and farms (Käsbohrer et al., 2000; Lomonaco et al., 2009). In Germany, *Salmonella* was isolated from 3.7% of the feces at seven slaughterhouses in 1996 (Käsbohrer et al., 2000). In Italy, 9.33% of the feces were positive when examined using polymerase chain reaction (Lomonaco et al., 2009). In this study, the *Salmonella* prevalence was relatively low level (2.5%), and is markedly lower than that reported (21%) in another study conducted in Korea (Suh et al., 2005). The reason for the discrepancy of the Korea-based studies may be the randomly selection of pigs in the present study versus the deliberate selection of pigs with diarrhea or pigs from herds with a history of diarrhea. In addition, pig farm hazard analysis critical control point (HACCP) program, which only began in November 2006, has improved the sanitary conditions of pig rearing in Korea, likely also reflected in the decreased prevalence rate of *Salmonella*.

S. typhimurium and *S. derby* have been reported as the predominant serotypes in Korea (Kim et al., 2007; Suh et al., 2005). However, presently, *S. rissen* was the most common serotype (52.38%). This may reflect an evolving serotype prevalence in Korea, or the more selective introduction of *S. rissen* into slaughterhouses.

In Europe and America, *S*. *rissen* is an uncommom serotype (McDowell et al., 2007; Käsbohrer et al., 2000; Mainar-Jaime et al., 2008; Lomonaco et al., 2009). On the other hand, similar, to the present study, *S. rissen* was the most common serotype in a report from Thailand (Padungtod et al., 2006). Recently, the prevalence of *S. rissen* infections and isolation of this serovar from pork products in Thailand has been increasing (Hendriksen et al., 2008). The predominant serotype in Thailand was similar to that in this study. Particularly, it is a serotype of *Salmonella* found most frequently among pig farm workers (Bangtrakulnonth et al., 2004). In Korea, a considerable number of foreign workers are currently employed on farms. To infer the cause of the introduction and change of *Salmonella* serotype, an investigation about *Salmonella* infections of farm workers will need to be done.

Antimicrobials have long been used for therapy and prevention of disease and growth promotion in pig production system. In particular, antimicrobial agents used as feed additives may have a negative effect on antimicrobial susceptibility. In Denmark, greater amounts of antimicrobial agents were consumed until 1995 in feed for growth promotion than for therapy of diseases. This practice may have decreased the therapeutic efficacy of antibiotics because of increased resistance (Aarestrup, 2005). In Canada, antimicrobial agents used as feed additives in finisher pigs were associated with resistance of *E. coli* (Varga et al., 2009). Antimicrobial resistance of bacteria in food animals could decrease the efficacy for treatment of animals and increase the potential risk in human health.

In antimicrobial susceptibility testing, highest prevalence of resistance was observed with tetracycline in both *Salmonella* (76.19%) and *E. coli* (87.11%), echoing results from Canada, although the degree of resistance to tetracycline was lower in Canadian isolates of *Salmonella* (43.4%) and *E. coli* (79.4%) (Varga et al., 2008b). In Korea, the resistance of *Salmonella* to tetracycline was 67.6% in 2005 (Suh et al., 2005) and that to oxytetracycline was 100% in 2007 (Kim et al., 2007). The amount of tetracycline used in the pig farming accounts for roughly 50% of the total amount of this antimicrobial agent given to pigs (Ha et al., 2003). Overuse of tetracycline has increased resistance to tetracycline in Korea and elsewhere (McDowell et al., 2007; Lomonaco et al., 2009). Tetracycline should not be expected to be a reliable preventative or curative strategy for salmonellosis.

The resistance of *E. coli* to chloramphenicol was presently rather high at 66.24%. Chloramphenicol is prohibited from being injected into food animals in Korea and other countries; still, the resistance rate is high (Varga et al., 2008a; Teshager et al., 2000). This may be partly due to co-resistance by use of dihydrostreptpmycin and trimethoprim (Harada et al., 2006). In Canada, the resistance of *E. coli* to sulfamethoxazole/trimethoprim was reported to be 6.4% (Varga et al., 2008a), in marked contrast to the resistance rate of 51.92% in the present study. In Korea, about 1000 tonnes of this class of sulfonamides are used in pig production, constituting the second largest portion among antimicrobial agents (Ha et al., 2003). So, resistance of sulfamethoxazole/trimethoprim in Korea has been high.

Presently, resistance to ampicillin/sulbactam and polymyxin B was low in both *Salmonella* and *E. coli*. Resistance to ampicillin/sulbactam was lowest in *Salmonella* (4.76%). In cases where ampicillin has been used as a single drug, resistance can be very prevalent, reached 92.98% in one study (Kim et al., 2007). Sulbactam plays a role impeding the enzyme that breaks down ampicillin (Harvey and Champe, 2009).

So, the resistance of ampicillin/sulbactam was noticeably low. Resistance to polymyxin B in the *E. coli* isolates was lowest (0.52%). In contrast, it is generally considered that susceptibility to polymyxin of *E. coli* is high (Aarestrup et al., 2008).

No significant association was found between the resistance patterns of the two bacteria. Yet, in both *Salmonella* and *E. coli*, relatively high frequencies of resistance were observed to antimicrobial agents commonly used in Korea (tetracycline, chloramphenicol, and sulfamethoxazole/trimethoprim).

In this study, a wide range of isolates presented multi-antimicrobial resistance. Fourteen (66.66%) of 21 *Salmonella* isolates and 611 (78.72%) of 776 *E. coli* isolates showed resistance to more than one antimicrobial. In *E. coli*, the most common resistance pattern was TE-SXT-K-C pattern (22.81%), reflecting the predominant use of these antibiotics in pig production.

Conclusion

This study has reported the *Salmonella* prevalence of finisher pigs at the lairage of all authorized slaughterhouses in Korea. The study has also shown the actual condition of antimicrobial resistance in *Salmonella* and *E. coli* from pigs' feces. It could be the essential database to lay out a proper antibiotic usage scheme. In antimicrobial susceptibility testing, except for several new drugs, high resistance was the norm.

Antibiotic resistance of tetracycline reached a serious level and resistance to chloramphenicol, which has been prohibited for use in food animals for an appreciable time, was still high. Moreover, most isolates displayed resistance to more than one antimicrobial. It is considered that multi-resistance of *E. coli* and *Salmonella* is at the serious level in pigs in Korea. Hence, a program of continuous surveillance and institutional guidelines with regard to animal antibiotic usage are prudent recommendations.

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