Salmonella Typhimurium Isolated from Healthy Pigs and Their Ability of Horizontal Transfer of Multidrug Resistance and Virulence Genes

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Salmonella is the most common food-borne pathogen worldwide. Rapid dissemination of multidrug-resistant strains, in particular from animal origins, is a growing concern to human and animal health, and it is mostly attributed to conjugative DNA exchange in the intestinal tract of food animals. To understand the potential role of pigs as a reservoir for antimicrobial-resistant Salmonella, we isolated Salmonella from slaughtered pigs and examined their drug resistance and gene transfer ability. We collected fecal and carcass swabs from 104 healthy pigs at an abattoir in Obihiro, Hokkaido, Japan. A total of 15 Salmonella strains were isolated, the most common serotypes being S. Typhimurium (7/15), S. Derby (2/15), S. Southampton (2/15) and S. O4:d:- (4/15). All of S. Typhimurium isolates were resistant to at least one of five antibiotics (ampicillin, kanamycin, sulfisoxazole, tetracycline and streptomycin). Pulsed-field gel electrophoresis (PFGE) profiles after XbaI and BlnI digestion were analyzed. S. Typhimurium isolates from 3 farms located in different regions clustered together and showed genetic relatedness. In conjugation experiments, one multidrug-resistant S. Typhimurium isolate showed the ability to transfer not only antibiotic resistance genes but also virulence genes such as spvABC to recipient bacteria. These results suggest that the spread of S. Typhimurium had occurred in pig farms and that asymptomatic Salmonella-infected pigs should be considered as a significant source of antibiotic-resistant bacteria.

Key words: Salmonella; pig; horizontal gene transfer; multidrug resistance

INTRODUCTION

Salmonella has been recognized as a major food-borne pathogen, and its contamination of animal products is a significant source of human salmonellosis. Resistance to antimicrobial agents within nontyphoidal Salmonella is considered a serious problem in the world. In the past decade, an obvious increase in overall antimicrobial-resistant Salmonella from 20% to 30% in the early 1990s to as high as 70% in the 2000s has been reported (29). In Japan, antibiotic-resistant Salmonella has also spread widely (1, 8). The extensive use of antimicrobials in humans and animals has led to an increase in multidrug-resistant Salmonella strains, especially S. Typhimurium DT 104, which was first recognized in the United Kingdom in 1984 and is now distributed throughout the world (11).

The role of food animals as a reservoir of multidrug-resistant Salmonella has become an issue of concern. The majority of Salmonella infections in food animals are asymptomatic. In addition, antibiotic resistance genes have been shown to accumulate on mobile genetic elements such as plasmids and transposons, leading to a condition where multidrug resistance phenotypes can be transferred horizontally to recipient bacteria. To study the potential role of pigs as a reservoir for antimicrobial resistant Salmonella, we determined the distribution of antibiotic-resistant Salmonella in slaughtered pigs at a local swine slaughterhouse, and analyzed the transfer ability of Salmonella isolates by in vitro and in vivo conjugation assays.

MATERIALS AND METHODS

Sampling

Feces and carcass samples were collected from 104 healthy slaughtered pigs, at a local abattoir in December 2006. The pigs had originated from 16 farms around Obihiro, Hokkaido, Japan. After slaughter, feces were taken from the rectal tract and swabbing was done on squares of 10.0 cm on the back side near the anus and shoulder areas using sterile cotton plugs (Eiken
Chemical, Tokyo, Japan) pre-moistened with sterile saline and transferred to 10 ml of sterile saline. The swab samples were collected after the carcasses had been washed using water rinses with sanitizing sprays. All samples were collected aseptically and transported to the laboratory and stored at 10°C until tested.

Isolation and identification
Each 0.1 g of feces or 1-ml portion of swab suspension was mixed with 0.9 ml or 9 ml of Buffer Peptone Water (primary Salmonella pre-enrichment broth; Oxoid, Hampshire, UK), respectively, followed by incubation at 37°C for 18 hr. Then, 0.1 ml of the culture was inoculated to 10 ml of Rappaport Vassiliadis soya peptone broth (secondary Salmonella enrichment broth; Kanto Chemical, Tokyo, Japan) and incubated at 42°C for 24 hr, after which an aliquot of the culture was spread on Deoxycholate Hydrogen sulfide Lactose agar (DHL; Eiken Chemical) and incubated at 42°C for 24 hr, respectively, followed by incubation at 37°C for 18 hr. Bacterial colonies were suspended in 200 µl of sterilized distilled water (OD 610 nm=0.5–0.6). The suspended cells were embedded with 200 µl of 1% agarose (Certified Megabase Agarose; Bio-Rad, Hercules, CA) in plugs. The cell-containing plugs were lysed in buffer consisting of 1 mg/ml proteinase K with 1% N-lauroylsarcosine in 0.5 M EDTA, pH 8.0. Cells were lysed overnight in a water bath at 50°C with gentle shaking. After lysis, the plugs were washed twice with sterile 4 mM Pefabloc SC (Roche Diagnostics, Manheim, Germany) in 1 ml of TE buffer for 20 min at 50°C with gentle agitation in a water bath to inactivate proteinase K (Wako Pure Chemical, Osaka, Japan) and remove excess reagents and cell debris from the lysed plugs; then, they were washed once with 3 ml of TE. DNA was digested with restriction enzymes XbaI and BlnI (Boehringer Mannheim, Indianapolis, IN), overnight in a water bath at 37°C. The plugs were then placed in a 1% agarose gel (Pulsed field-certified agarose). Restriction fragments were separated by electrophoresis in 0.5 × TBE buffer at 14°C for 21 hr using a Chef-DRII (Bio-Rad) with pulse times of 2.2 to 54.2 sec. The gels were stained with ethidium bromide and destained in water. The DNA bands were visualized with UV transillumination.

Antibiotic resistant test
To clarify antibiotic resistance patterns in the isolates, disk diffusion susceptibility tests (24) using Sensi-Disc (Japan Becton Dekinson, Tokyo, Japan) were performed according to the supplier’s instructions using fourteen antibiotics of human and veterinary significance: ampicillin (Am) 10 µg, chloramphenicol (Cm) 30 µg, kanamycin (Km) 30 µg, nalidixic acid (Nal) 30 µg, streptomycin (Sm) 10 µg, sulfisoxazole (Su) 250 µg, tetracycline (Tc) 30 µg, gentamicin (Gm) 10 µg, ciprofloxacin (Cip) 5 µg, ofloxacin (OFX) 5 µg, levofloxacin (LVX) 5 µg, cephalothin (CF) 30 µg, cefotaxime (CTX) 30 µg and cefepime (Fep) 30 µg. Inhibitory zones of the growth were measured and interpreted either as recommended by the supplier or the criteria of the National Committee for Clinical Laboratory Standards (NCCLS), except for intermediate and sensitive isolates which were grouped together.

Pulsed-field gel electrophoresis (PFGE)
To assess genetic relatedness in all Salmonella isolates, PFGE was performed. Plug preparation, restriction digestion, electrophoresis conditions and staining of gels were carried out according to the PulseNet standardized PFGE protocol (http://www.cdc.gov/pulsenet/protocols.htm) with the following modifications. Bacteria were grown on Trypticase Soy Agar (TSA) at 37°C for 18 hr. Bacterial colonies were suspended in 200 µl of sterilized distilled water (OD 610 nm=0.5–0.6). The suspended cells were embedded with 200 µl of 1% agarose (Certified Megabase Agarose; Bio-Rad, Hercules, CA) in plugs. The cell-containing plugs were lysed in buffer consisting of 1 mg/ml proteinase K with 1% N-lauroylsarcosine in 0.5 M EDTA, pH 8.0. Cells were lysed overnight in a water bath at 50°C with gentle shaking. After lysis, the plugs were washed twice with sterile 4 mM Pefabloc SC (Roche Diagnostics, Manheim, Germany) in 1 ml of TE buffer for 20 min at 50°C with gentle agitation in a water bath to inactivate proteinase K (Wako Pure Chemical, Osaka, Japan) and remove excess reagents and cell debris from the lysed plugs; then, they were washed once with 3 ml of TE. DNA was digested with restriction enzymes XbaI and BlnI (Boehringer Mannheim, Indianapolis, IN), overnight in a water bath at 37°C. The plugs were then placed in a 1% agarose gel (Pulsed field-certified agarose). Restriction fragments were separated by electrophoresis in 0.5 × TBE buffer at 14°C for 21 hr using a Chef-DRII (Bio-Rad) with pulse times of 2.2 to 54.2 sec. The gels were stained with ethidium bromide and destained in water. The DNA bands were visualized with UV transillumination.

PCR
The primers used in this study to detect resistance genes for Am, Km and Tc are shown in Table 1 (12). Aminoglycoside phosphotransferase (aphA1-lab) which encodes Km resistance, β-lactamase TEM (blaTEM) which encodes Am resistance and Te resistance, and tetA(A), tetA(B) and tetA(G) were amplified. The PCR amplification was carried out in a mixture of 25 µl containing 1 × Ex Taq Buffer (Mg²⁺-plus), 0.625U Ex Taq DNA polymerase (Takara Bio, Shiga, Japan), 0.2 mM dNTPs, 0.2 µM primers and 1 µl of genomic DNA (100 ng/µl). DNA was extracted by a MORAX-Extract kit following the supplier’s protocol (Kyokuto Pharmaceutical, Tokyo, Japan). DNA concentrations were measured by spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, Wilmington, DE). The PCR conditions were performed with the first denaturation cycle at 95°C for 5 min, followed by 30 cycles of 95°C for
MOLECULAR CHARACTERIZATION OF SWINE Salmonella ISOLATES

1 min, 54°C for 1 min (for aphA1-Iab and blaTEM) or 58°C for 1 min [for tetA(A), tetA(B) and tetA(G)] and 72°C for 1 min with a final extension at 72°C for 7 min.

Conjugate experiment in vitro

To clarify whether or not S. Typhimurium isolates have the ability to transfer their resistance genes, liquid and filter mating procedures were performed in vitro (21) using rifampicin-resistant Escherichia coli C600 (RifR) and Nal-resistant S. Dublin (NalR) derivatives as recipient strains. S. Typhimurium isolate No.7 was used as the donor strain. L-broth (Takara Bio) was used to cultivate the donor and recipient strains. Aliquots, 0.9 ml, of the donor (1.82 × 10⁹ CFU/ml) were mixed either with 0.1 ml of the recipients, E. coli C600 (RifR) culture (2.31 × 10⁸ CFU/ml) or S. Dublin (NalR) culture (1.02 × 10⁹ CFU/ml). For broth mating, the mixtures were incubated at 37°C for 16 hr. For filter mating, the mixtures were filtered by membrane (0.2 μm) to collect bacterial cells; then, the filter was placed with the bacteria side down on a TSA plate and incubated at 37°C for approximately 16 hr. Bacterial cells were harvested from the filter, and suspended at a final volume of 1 ml saline. The bacterial culture was then serially diluted and plated on DHL plates with Rif (100 μg/ml) and appropriate antibiotics to select E. coli C600 transconjugants. Control mice received either the donor or recipient only. Frequency was calculated as transconjugants per recipient per mouse.

Identification of S. Typhimurium DT104 by PCR

The PCR amplification of an internal segment of the 16S-to-23S spacer region of bacterial rRNA genes was used to identify S. Typhimurium DT104 as previously described (27).

RESULTS

Prevalence of Salmonella in slaughter pigs

A total of 15 Salmonella isolates were isolated from feces and carcass samples collected from 104 healthy pigs. The most common serotype was S. Typhimurium (46.7%), followed by Salmonella (O4:d–) (26.7%), S. Derby (13.3%), and S. Southampton (13.3%) (Table 2). These Salmonella, except for one S. Typhimurium isolate from a carcass, were isolated from stool samples. S.
Typhimurium was isolated from 4 farms. *Salmonella* (O4:d:–) was isolated twice from Farm II at an one-week interval. Moreover, *Salmonella* was isolated from 9 out of 15 pigs that originated from Farm II, indicating that most of pigs at that farm were infected with *Salmonella*.

**Antimicrobial resistance and virulence phenotypes of *Salmonella* isolates**

The disk diffusion susceptibility tests were performed to clarify antibiotic resistance patterns in the isolates (Table 2). All *S. Typhimurium* isolated in this study were resistant to Su. Five out of seven *S. Typhimurium* isolates were resistant to more than two antibiotics while the other serotypes were sensitive to all tested antibiotics. PCR was performed to identify *S. Typhimurium DT104* (27), but DT104 was not detected in this study (data not shown). As shown in Table 2, virulence gene phenotypes were also determined by PCR. All *Salmonella* isolates were found positive for the invA gene. However, only *S. Typhimurium* strains were positive for spvABC. Three and four of seven *S. Typhimurium* isolates were positive for pefA and rck genes, respectively (Table 2).

**PFGE patterns of *Salmonella* isolates**

Six types of PFGE patterns after *XbaI* digestion (X1, X2a, X2b, X3, X4 and X5) and 7 types of PFGE patterns after *BlnI* digestion (B1, B2a, B2b, B2c, B3, B4 and B5) were observed (Table 2 and Fig.1). Types X2a and X2b resembled each other and differed by a single band. Types B2a, B2b and B2c resembled each other. Single band differences between B2a and B2b, and between B2a and B2c was observed. Two band differences between B2b and B2c was observed. Among *S. Typhimurium* isolates, 3 unique PFGE patterns were observed after *XbaI* digestion, and 4 patterns after *BlnI* digestion (Fig. 1). For other serotypes, PFGE patterns were matched among isolates of the same serotype. The *S. Typhimurium* isolate No.1 showed the same PFGE pattern with No.11 and No.14 and carried the same virulence genes although their drug resistance patterns, isolation farms and dates were different with each other (Table 2). Similarly, PFGE patterns of *S. Southampton* No.10 and No.15, which were isolated from Farm II and Farm V, respectively, were almost identical with each other (Table 2). In Farm II, 4 (2 and 2) *Salmonella* strains (O4:d:–) with the same PFGE pattern were isolated at an one-week interval (Table 2). These results suggest that *Salmonella* with a similar genetic background might have already spread among pigs and farms in the tested area.

**Gene transfer of antibiotic resistance genes and virulence genes**

Next, we clarified whether or not *S. Typhimurium* isolates have the ability to transfer their resistance genes to other bacteria. The liquid mating procedure (21) was performed at 37°C in *E. coli* C600 as the recipient strain (Table 3). Among five multidrug-resistant *S. Typhimurium* isolates, only *S. Typhimurium* No.7 showed the ability to transfer antibiotic resistance in *E. coli* C600 recipient cell, respectively (Table 3). We also observed the successful transfer of antibiotic resistance genes from *S. Typhimurium* No. 7 to

### Table 2. Characterization of *Salmonella* strains isolated from stools and carcasses of slaughtered pigs

| *Salmonella* strain | Isolation date | Farm | Source | Antibiotic resistance(a) | Virulence genes(b) | PFGE pattern | Ability of gene transfer |
|--------------------|----------------|------|--------|--------------------------|-------------------|-------------|-------------------------|
| S. Typhimurium No. 1 | 2006/12/14 | I | Stool | CmSu | invA + spvA + spvB + spvC + rck + | X1 B1 | – |
| S. Typhimurium No. 2 | 2006/12/14 | II | Carcass | AmKmSuTc | invA + spvA + spvB + spvC | X2a B2a | – |
| S. Typhimurium No. 3 | 2006/12/14 | II | Stool | AmKmSuTc | invA + spvA + spvB | X2b B2b | – |
| S. Typhimurium No. 4 | 2006/12/14 | II | Stool | AmKmSuTc | invA + spvA + spvB | X2b B2c | – |
| S. Typhimurium No. 5 | 2006/12/14 | II | Stool | AmKmSuTcSm | invA + spvA + spvB | X2a B2c | + |
| S. Typhimurium No. 6 | 2006/12/15 | III | Stool | Su | invA + spvA + spvB | X1 B1 | NT |
| S. Typhimurium No. 7 | 2006/12/15 | IV | Stool | Su | invA + spvA + spvB | X1 B1 | NT |
| Salmonella(O4:d:–) No. 3 | 2006/12/14 | II | Stool | – | invA + spvA + spvB | X3 B3 | NT |
| Salmonella(O4:d:–) No. 5 | 2006/12/14 | II | Stool | – | invA + spvA + spvB | X3 B3 | NT |
| Salmonella(O4:d:–) No. 8 | 2006/12/21 | II | Stool | – | invA + spvA + spvB | X3 B3 | NT |
| Salmonella(O4:d:–) No. 9 | 2006/12/21 | II | Stool | – | invA + spvA + spvB | X3 B3 | NT |
| S. Southampton No. 10 | 2006/12/21 | II | Stool | – | invA + spvA + spvB | X4 B4 | NT |
| S. Southampton No. 15 | 2006/12/21 | V | Stool | – | invA + spvA + spvB | X4 B4 | NT |
| S. Derby No. 12 | 2006/12/15 | VI | Stool | – | invA + spvA + spvB | X5 B5 | NT |
| S. Derby No. 13 | 2006/12/15 | VI | Stool | – | invA + spvA + spvB | X5 B5 | NT |

NT: Not tested. a)14 antibiotics were tested to determine multidrug resistance. b) +; detected by PCR, –; not detected by PCR.
MOLECULAR CHARACTERIZATION OF SWINE Salmonella ISOLATES

a different serotype, S. Dublin (data not shown). Next, we examined whether the resistance genes were transferred in vivo (Table 3). Km\(^{r}\) transconjugants were detected at frequencies of 0.24 \(\times\) 10\(^{-7}\) cells per E. coli cell per mouse at 12 hr post inoculation, but Am\(^{r}\) and Tc\(^{r}\) transconjugants were not detected (Table 3), suggesting that, when antibiotic-resistant bacteria exist in normal intestinal tracts, antibiotic sensitive bacteria would acquire resistance without any selection pressures.

Antibiotic resistance of in vitro and in vivo transconjugants was tested (Table 4). Surprisingly, in vitro transconjugants selected for either Am\(^{r}\) or Tc\(^{r}\) showed resistance to Am, Tc and Km. The presence of the corresponding resistance genes, bla\(_{\text{TEM}}\) gene encoding \(\beta\)-lactamase TEM responsible for Am resistance, aph\(_{1}\)-lab gene encoding aminoglycoside phosphotransferase responsible for Km resistance, and tetA(A) gene encoding Tc efflux protein, in the transconjugants was also detected by PCR (Fig. 2). In vitro and in vivo transconjugants selected for Km\(^{r}\) showed resistance to Km but not to Am or Tc, and only the aph\(_{1}\)-lab gene was detected among them.

To ascertain if the virulence genes in the donor S. Typhimurium were transferred together with the antibiotic resistance gene, PCR analyses for the invA and spvABC genes was performed. As shown in Table 4 and Fig. 3, the donor strain, S. Typhimurium No.7, possessed the invA and spvABC genes while the recipient strain E. coli possessed none of them. Transconjugants selected for Am\(^{r}\) or Tc\(^{r}\), except for only two Am\(^{r}\) transconjugants, possessed the spvABC but not the invA gene. This indicates that the virulence genes, spvABC genes in S. Typhimurium were horizontally transferred to E. coli. The results and those of the PCR analyses for antibiotic resistance genes indicate that the antibiotic resistance and virulence genes were horizontally transferred to other bacteria together. However, the transconjugants which were collected from Km selection of both in vitro and in vivo conjugation were all negative for the virulence genes.

**DISCUSSION**

In this study, Salmonella was examined in slaughtered pigs from local farms. Four serotypes namely, S. Typhimurium, S. Southampton, S. Derby and \(O4:d:-\), were isolated. The most frequent serovar was S. Typhimurium. Our findings were in agreement with other studies which have found S. Typhimurium to be one of the most predominant serotypes isolated from animals and humans all over the world (8, 15, 18, 22, 30, 31). \(O4:d:-\) was also isolated from pigs from Farm II at an interval of 1 week. Since Salmonella group O4 is one of the dominant serovars of Salmonella isolates from pigs in mainland Japan (2, 3), this serotype might be widely distributed in Hokkaido. The European Food

| Table 3. In vitro and in vivo gene transfer frequencies |
|------------------------------------------------------|
| Conjugation experiment | Antibiotics used for selection | Transfer frequency \(^a\) |
|-------------------------|--------------------------------|--------------------------|
| in vitro                | Am                             | 0.52 \(\times\) 10\(^{-3}\) |
|                         | Km                             | 0.18                     |
|                         | Tc                             | 0.41 \(\times\) 10\(^{-3}\) |
| in vivo                 | Am                             | –                        |
|                         | Km                             | 0.24 \(\times\) 10\(^{-7}\) |
|                         | Tc                             | –                        |

\(^a\) Transfer frequency of in vitro and in vivo experiments was calculated as described in the text.
Safety Authority (EFSA) analysis has revealed some similarities between the *Salmonella* types most frequently reported in humans and those found in slaughtered pigs, implying that pigs and pig meat do contribute to *Salmonella* infections in humans (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812-1178620776028.htm).

Epidemiological surveillance of antimicrobial-resistant *Salmonella* has become necessary for effective treatment and prediction of the occurrence of resistant populations of prevalent serotypes. This study screened the antibiotic resistance patterns of all *Salmonella* isolates. The resistance profiles showed that only *S. Typhimurium* isolates were resistant to antibiotics. Drug-resistant *S. Typhimurium* could easily be detected, especially, multidrug-resistant phage type DT104, which has become the dominant isolate (4, 9, 11). This finding is additional evidence that *S. Typhimurium* has become a drug-resistant strain. The multidrug-resistant strain *S. Typhimurium* DT104 was initially characterized as having chromosomally located genes for resistance to Am, Cm, Sm, sulfonamides and Tc, but strains with additional resistance or decreased susceptibility to Gm, trimethoprim, and/or fluoroquinolones have been reported in recent years (4). In this study, multidrug-resistant *Salmonella* was isolated from pigs, however

Table 4. *In vitro* and *in vivo* transfer of antibiotic resistance and virulence genes

| Bacterial strains | Antibiotics used for | Antibiogram\(^a\) | Resistance genes\(^b\) | Virulence genes\(^c\) | No. of strains |
|-------------------|----------------------|-----------------|-----------------|-----------------|----------------|
| Donor *S. Typhimurium* No. 7 | Am, Km, Tc | R, R, R | +, +, + | invA, spvA, spvB, spvC | 20 |
| Recipient *E. coli* K12 C600 | Am, Km, Tc | R, R, R | +, +, + | invA, spvA, spvB, spvC | 2 |

\(^a\) R; resistant, S; sensitive.  \(^b\), \(^c\) +; detected by PCR, –; not detected by PCR.

**Fig. 2.** *In vitro* horizontal gene transfer of antimicrobial resistance genes. The liquid mating procedure was performed at 37°C *in vitro* using *S. Typhimurium* No. 7 as a donor strain and Rifr *E. coli* K12 C600 as a recipient strain. Transconjugants were selected on DHL agar with appropriate selective antibiotics: Rif 100 μg/ml, Am 100 μg/ml, Km 50 μg/ml, Tc 30 μg/ml, Cm 30 μg/ml, and Km 50 μg/ml. The *bla*\(_{TEM}\), *aphA1-lab* and *tetA*\((A)\) genes of transconjugants were analyzed by PCR. Lane: 1, *S. Typhimurium* No.7 (donor); 2, *E. coli* C600 (recipient); 3, transconjugant selected as AmR; 4, transconjugant selected as KmR; TcR.

**Fig. 3** *In vitro* horizontal gene transfer of virulence genes. The *in vitro* conjugation was performed as described in Material and Methods. The *invA*, *spvA*, *spvB* and *spvC* genes of transconjugants were analyzed by PCR. Lane: 1, *S. Typhimurium* No.7 (donor); 2, *E. coli* C600 (recipient); 3 and 4, transconjugants selected as AmR; 5, transconjugant selected as KmR; TcR.
DT104 was not detected. DT104 has been detected in humans and cattle, however it appears not to be widely distributed among pigs yet.

The genetic relatedness among all *Salmonella* isolates was examined by PFGE patterns. The results of this study suggest that *Salmonella* with a similar genetic background might have spread among pigs and farms in Hokkaido. *S. Typhimurium* No.2 which was isolated from a carcass, but not from its feces, presented a similar genotype and resistance phenotype as the three *S. Typhimurium* isolates from other pig feces from animals slaughtered on the same day and from the same farm (Table 2). *Salmonella* spp. is often found in the intestinal tracts of domestic animals. During slaughtering and meat processing, *Salmonella* may contaminate carcasses, meats and the environment, and will disseminate to humans and animals. A large-scale epidemiological survey on the environments of slaughter-houses as well as pig farms, and workers would clarify this point.

The presence of *aphA1-lab*, *blaqTEM*, and *tetA(A)* genes which encode resistance to Km, Am and Tc, respectively, among *S. Typhimurium* isolates from slaughtered pigs is alarming. Because antibiotics are necessary in the human and veterinary medical fields, the appearance of drug resistance genes in pathogens is a big obstacle to the treatment of infectious diseases. In this study, Am, Km and Tc resistance genes of *S. Typhimurium* No.7 were transferred to *E. coli* C600 and *S. Dublin* *in vitro*; and we found that the transfer frequencies to *S. Dublin* were much higher than to *E. coli* C600, raising the possibility that intraspecies transfer of resistance genes might be easier than interspecies. In addition, the Km' gene was also transferred to *E. coli* C600 during transient colonization in the GI tract of ICR mice in the absence of selective pressure. Even though the colonization was observed to be transient, these studies provide evidence that the GI tract represents not only a staging area for the expansion of pathogenic isolates, but also an environment in which the transfer of resistance genes to commensal strains could give rise to new resistant isolates. This suggests that if antibiotic-resistant bacteria exist in the intestinal tract, then various new antibiotic-resistant bacteria might also be generated without any selection pressure. Since antibiotic therapy is essential in the medical and veterinary fields, the appearance of drug-resistant pathogens is a big obstacle to the treatment of infectious diseases.

*S. Typhimurium* isolated from pigs in this study were highly pathogenic as we found the presence of the *spv* operon together with *invA*, *rek* and *pefA* genes. *S. Typhimurium* is known to harbor a serovar-specific virulence plasmid that contains the *spv* operon (7). It has been suggested that *spv* genes are important for pathogenesis in humans as *spv*-carrying strains dominate among clinical isolates from patients with non-typhoidal bacteraemia (23, 28). Non-typhoidal salmonellosis is characterized by gastroenteritis, and is associated with intestinal inflammation and diarrhea. The *spv* genes increase the severity of systemic disease (20). The *spv* operon consists of the transcriptional regulator *spvR* and four structural genes *spvABCD*. The SpvR protein promotes *spvABCD* gene expression (17). The SpvB protein is an ADP ribosyltransferase that modifies actin and destabilizes the cytoskeleton of infected cells, and it appears to be primarily responsible for the *spv* virulence phenotype (19). The other virulence genes detected in this study are also important for *Salmonella* pathogenesis: *invA* encoding effector protein, *rek* gene encoding outer membrane protein required for both serum resistance and cell invasion, and *pefA* gene encoding major fimbrial subunit (10). We demonstrated that *S. Typhimurium* isolates of pig origin could transfer not only antibiotic resistance profiles but also virulence genes to non-pathogenic *E. coli*. *E. coli* is a commensal bacterium of the facultative anaerobic colonic microflora. Some strains of *E. coli* which may have acquired virulence determinants, during the molecular evolution process, cause intestinal or systemic diseases (16). Thus, the horizontal acquisition of virulence factors implies significant clinical and food safety issues. Understanding of the mechanism of horizontal gene transfer may provide a strategy to reduce the potential risk of the dissemination of these genes.

In conclusion, the main findings of this study were as follows: 1) all of *S. Typhimurium* isolates from healthy pigs had antimicrobial resistance; 2) one *S. Typhimurium* isolate of pig origin could transfer not only antimicrobial resistance genes but virulence genes to commensal bacteria such as *E. coli*. These results suggest that *Salmonella*-derived virulence and antibiotic resistance genes could be transferred inter- or intra-species via the intestinal tract of reservoir animals. Overall, our data have important implications for public health and food safety, and provide useful information on the dissemination of pathogens with antimicrobial resistance and virulence genes in food animals.

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MOLECULAR CHARACTERIZATION OF SWINE *Salmonella* ISOLATES

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