Prevalence and Characterization of Multidrug-Resistant (Type ACSSuT) 
Salmonella enterica Serovar Typhimurium Strains in Isolates from Four 
Gosling Farms and a Hatchery Farm

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Salmonella enterica serovar Typhimurium strains of phage types DT104 and U302 are often resistant to 
ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (the ACSSuT resistance type) and 
are major zoonotic pathogens. Increased consumption of goose meat may enhance the risk of transferring S. enterica serovar Typhimurium and other enteric pathogens from geese to human due to the consumption of 
meats from infected geese or improper preparation of meats. Therefore, we characterized S. enterica serovar Typhimurium strains isolated from four goose farms (farms A, B, C, and D) and one hatchery farm (farm E) 
to determine the epidemic and genetic differences among them. Antibiotic susceptibility tests and multiplex 
PCR confirmed that 77.6% (52/67) of strains were ACSSuT strains isolated from farms A, C, and E. Antibiotic-
susceptible strains were isolated mostly from farm B, and no strain was observed in farm D. All ACSSuT 
strains harbored a 94.7-kb virulence plasmid and contained one 1.1-kb conserved segment identical to that of 
Salmonella genomic island 1. Four genotypes were determined among these S. enterica serovar Typhimurium 
isolates by pulsed-field gel electrophoresis analysis of XbaI-digested DNA fragments. Most isolates (85.29%; 
29/34) of major genotype Ib were ACSSuT strains isolated mainly from goslings of farm C and egg membranes 
of farm E, a hatchery farm, suggesting that S. enterica serovar Typhimurium strains in isolates from goslings 
might originate from its hatchery, from the egg membranes to the gosling fluff after hatching. Multiple phage 
types, types 8, 12, U283, DT104, and U302, were identified. In conclusion, geese were a reservoir of diverse 
multidrug-resistant (type ACSSuT) S. enterica serovar Typhimurium strains, and each farm was colonized with 
genetically closely related S. enterica serovar Typhimurium strains.

Outbreaks of salmonellosis caused by Salmonella enterica serovar Gallinarum result in great economic losses due to the high mortality of young chickens; however, S. enterica serovar Typhimurium appears to have a higher invasion capability than S. enterica serovar Gallinarum (11). As one of the most predominant Salmonella serovars isolated from poultry, S. enterica serovar Typhimurium can be transmitted vertically through 
eggs and horizontally from the contaminated eggs and meat to 
humans (1). In ducklings, 93% of Salmonella isolates are S. enterica serovar Typhimurium (30). The infected duckling may 
be dehydrated and emaciated, have difficulty breathing, and 
even die in opisthotonus. In the United Kingdom, S. enterica serovar Typhimurium and S. enterica serovar Enteritidis have been reported to cause septicaemia in young ducklings with mixed infection with other pathogens (18). In market-ready 
geese, S. enterica serovar Typhimurium is the major serovar 
isolated from 60% of fluids from carcass rinsings and 18.4% of 
cloacal swabs (25). In addition, S. enterica serovar Typhi-
murium also caused a few cases of chronic salpingitis in geese 
and ducks (6).

S. enterica serovar Typhimurium usually harbors a 94.7-kb virulence plasmid (pSTV) encoding the 8-kb spv operon (16) 
that is involved in survival in the macrophage and then assists 
in systemic infection (21). The ccdA/B addition system between the 
spv operon and RepFIB in pSTV (GenBank accession 
number AE006471) maintains plasmid stability (34). Since multidrug-resistant (type ACSSuT) phage type DT104 was dis-
covered in 1984 (35), a 43-kb multidrug resistance DNA region 
responsible for the ACSSuT type has been found and named 
Salmonella genomic island 1 (SGI1), which is inserted between 
the thdF and yidY genes and contains two conserved-segment 
(CS) regions (7). Due to the process of evolution by recombi-
nation, insertion, and deletion events, SGI1 variants have been 
found in several serovars and may enhance the virulence and 
dissemination of the host strain (27). Recently, we reported 
multidrug-resistant (type ACSSuT) S. enterica serovar Typhi-
murium phage types DT104, DT120, and U302 isolated from
human in Taiwan (13). To elucidate whether these multidrug-resistant *S. enterica* serovar Typhimurium phage types can also be isolated from goose, the prevalence of multidrug-resistant *S. enterica* serovar Typhimurium and the pSTV profile, genotyping, and phage typing of these goose isolates were analyzed. Despite the existence of phage types DT104 and U302 in human and goose isolates, other *S. enterica* serovar Typhimurium phage types, types 8, 12, and U283, were isolated from geese, which were not of human origin.

**MATERIALS AND METHODS**

**Collection and enrichment of samples.** The samples were taken from five different goose farms in Chiayi, Taiwan, from 2004 to 2005. The culled sick geese were detected by necropsy and laboratory examination on farm A and farm C according to methods described previously Zander et al. (40). A method employing cloacal swabbing was used to examine *S. enterica* serovar Typhimurium infection of 4-week-old geese with diarrhea from farms A and C and healthy birds from farms B, C, D, and E (Table 1). All broths and selected agars used for infection of 4-week-old geese from farms A and C and healthy according to methods described previously Zander et al. (40). A method em-

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**TABLE 1. Sampling locations, methods, and clinical conditions of geese used in the study**

<table>
<thead>
<tr>
<th>Farm [test date (yr/mo)]</th>
<th>Sampling method</th>
<th>Age</th>
<th>Clinical body condition</th>
<th>Test code</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (2002/6)</td>
<td>Necropsy</td>
<td>2 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sick</td>
<td>FAN</td>
<td>5</td>
</tr>
<tr>
<td>B (2003/11)</td>
<td>Cloacal swab</td>
<td>3 wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>CBC</td>
<td>6</td>
</tr>
<tr>
<td>C (2004/8–9)</td>
<td>Necropsy</td>
<td>2–5 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sick</td>
<td>FCN</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cloacal swab</td>
<td>1 wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>FAC1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cloacal swab</td>
<td>4 wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sick</td>
<td>FAC4</td>
<td>27</td>
</tr>
<tr>
<td>D (2004/8–9)</td>
<td>Cloacal swab</td>
<td>1 wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>FBC1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cloacal swab</td>
<td>4 wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>FBC4</td>
<td>0</td>
</tr>
<tr>
<td>E (2004/8–9)</td>
<td>Cloacal swab</td>
<td>0 days&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>HGC</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hatching egg membrane</td>
<td></td>
<td></td>
<td>HHE</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Hatching cabinet</td>
<td></td>
<td></td>
<td>HHC</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> These geese were sick at the time of clinical examination.

<sup>b</sup> These geese were healthy at the time of clinical examination.

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were selected on the other end of a paper bridge containing first-phase H antiserum across the culture medium. **Antibiotic susceptibility test.** Antimicrobial susceptibility was tested by a stan-

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**RESULTS**

*S. enterica* serovar Typhimurium infection in geese. The prevalence of *S. enterica* serovar Typhimurium was 7% (36 of 513 samples) for cloacal swab samples and 10.7% (16 of 150
TABLE 2. Antibiotic susceptibilities and distributions of the virulence plasmid of 67 S. enterica serovar Typhimurium strains from different farms

<table>
<thead>
<tr>
<th>Type of drug resistance</th>
<th>Total no. of isolates</th>
<th>CS region</th>
<th>pSTV (94.7 kb)</th>
<th>Sample designation(s) for isolation source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Presence</td>
<td>No. of isolates</td>
<td>Presence</td>
</tr>
<tr>
<td>ACSSuT</td>
<td>52</td>
<td>+</td>
<td>52</td>
<td>+</td>
</tr>
<tr>
<td>ACSSu</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>CSSu</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>SSu</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>S</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>None*</td>
<td>9</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>67</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

* These strains were isolated from CBC of farm B.

... samples) for hatching eggshell membranes. Although differing in prevalence among sampling sources, the highest isolation rate (22.9%; 27/118) was obtained in diseased sample FAC4 (C-FAC4) isolates and not in normal FAC1 (C-FAC1) isolates of farm C (Table 1). Also, no S. enterica serovar Typhimurium isolate was found in normal farm D and E-HHC as well as E-HHE samples (Table 1).

Antibiotic susceptibility and SGI analysis. After antibiotic susceptibility analysis by the disk diffusion method and multiplex PCR analysis, 77.6% (52/67 isolates) of S. enterica serovar Typhimurium were of the ACSSuT type (Table 2 and Fig. 1). Most ACSSuT strains were isolated from C-FAC4 (25/52 isolates) and E-HHE (15/52 isolates); however, these resistant strains were not found in B-CBC. Except for one strain that appeared in the 1.3- and 1.1-kb SGII-specific CS region, all ACSSuT strains contained the 1.1-kb SGII-specific CS region with identical sequences, which was not amplified from non-ACSSuT strains (Table 2).

Virulence plasmid, genotype, and phage types. S. enterica serovar Typhimurium usually harbors a 94.7-kb virulence plasmid. Here, all ACSSuT S. enterica serovar Typhimurium isolates (100%; 52/52 isolates) harbored pSTV, which is missing in three strains isolated from E-HHE (Table 2). To understand the genomic variation among these S. enterica serovar Typhimurium isolates, PFGE analysis of XbaI-digested fragments separated 58 strains into four major genotypes (Fig. 2). Genotype distributions varied among isolation sources, and the major genotype, genotype Ib (74%; 43/58 isolates), appeared only in E-HHE as well as C-FAC1 and C-FAC4 (Table 3). Almost all isolates showed identical genotypes, such as genotype II found only in B-CBC samples, or one major genotype at the same farm (Table 3). However, three genotypes (genotypes I, II, and IV) were observed in C-FAC4.

Phage typing further differentiated these strains and determined the existence of a DT104 strain. Although only seven of the tested strains were analyzed, five phage types, types 8, 12, DT104, U283, and U302, were obtained (Fig. 2). Among these genotypes, genotype Ib was further divided into three phage types, types 8, 12, and U302. In contrast, the same phage type (types 8 and 12) appeared in different genotypes. Moreover, similar to genotype distribution, multiple phage types were observed in C-FAC4 strains.

DISCUSSION

S. enterica serovar Typhimurium is widely distributed among diverse ranges of animals, and hence, the prevalence of the pathogen in poultry is not unexpected. In poultry, S. enterica serovar Typhimurium appeared to have a superior invasion capability compared to S. enterica serovar Gallinarum (11). Therefore, S. enterica serovar Typhimurium has been frequently found in young chickens, imported frozen chickens, ducklings, and geese in different countries (6, 18, 20, 25, 26, 28, 30). Although S. enterica serovar Typhimurium may not be a primary cause of disease, it could possibly cause septicemia in young ducklings with complications of other diseases (18). The association of S. enterica serovar Typhimurium with septicemia or diarrhea in young geese (farms A and C) and subclinical infection with S. enterica serovar Typhimurium (farm B) imply that systemic infection with S. enterica serovar Typhimurium with complications of other pathogens may cause the death of geese. Previous reports indicated that the 94.7-kb plasmid pSTV is involved in systemic infection (21). In the present study, most of the S. enterica serovar Typhimurium isolates collected from human and geese harbored pSTV (13) (Table 2), which carries the ccdAB addiction system (34), and the spv operon (21), both responsible for the existence of pSTV in S. enterica serovar Typhimurium and the enhancement of the systemic infection of S. enterica serovar Typhimurium in humans and animals, respectively.

Salmonella can cause food-borne illness in humans due to contaminated meats and/or eggs, where Salmonella localizes...
on the inner membranes of eggshells in isthmal secretions (8), and the inner membrane also provides a physical barrier to prevent the entry of \textit{Salmonella}. These studies revealed that \textit{S. enterica} serovar Typhimurium existed on the inner membrane of goose eggs, as previously reported for duck egg membranes (31), but not from cloacal swabs of 0-day-old goslings and the hatching cabinet (Table 1), which are the most frequently found sites of \textit{S. enterica} serovar Enteritidis and \textit{S. enterica} serovar Typhimurium contamination (5, 9, 24) and a useful target to evaluate \textit{S. enterica} serovar Typhimurium contamination (3, 4). In addition, egg contamination by salmonellae can occur through contact with \textit{Salmonella}-contaminated equipment, personnel, and the environment and infected wild birds and rodents (14, 15), followed by the entry of \textit{Salmonella} into hatching eggs in a hatchery. The transmission of \textit{S. enterica} serovar Typhimurium from the hatchery to goslings was found in farm A since the goslings originated from a hatchery with high levels of contamination of \textit{S. enterica} serovar Typhimurium on eggshell membranes (Tables 1 and 3). The XbaI-digested PFGE profile indicated that prolonged exposure to the external environment such as a longer feeding period (genotype Ib for C-FAC1 versus genotypes Ia, Ib, II, and IV for C-FAC4) can increase the prevalence of diverse multidrug-resistant \textit{Salmonella} strains in geese (Fig. 2 and Tables 2 and 3). In addition, identical genotypes in each farm, such as genotype IIIb in A-FAN and genotype Ib in C-FAC1 and E-HHE, and genotype differences among farms (Table 3) suggest that each farm was colonized with genetically closely related \textit{S. enterica} serovar Typhimurium strains.

Generally, adequate sanitation can reduce the prevalence of \textit{Salmonella}. For example, proper egg washing may minimize the problem of \textit{Salmonella} contamination on outer eggshells (5, 22) and transmission from incubator to hatchers (9, 10). However, improper washing or rinsing of eggs, such as processing at lower temperatures, may increase the entrance of \textit{S. enterica} serovar Typhimurium and \textit{S. enterica} serovar Enteritidis into egg contents (22), and the washing process cannot remove \textit{Salmonella} contamination in the inner membrane and egg contents. Therefore, a vaccine is needed to prevent \textit{S. enterica} serovar Typhimurium and \textit{S. enterica} serovar Enteritidis infection.

Since the discovery of a multidrug-resistant ACSSuT \textit{S. enterica} serovar Typhimurium DT104 strain in United Kingdom (35), this strain has spread around the world, and it is prominently distributed in Europe and Northern America (17, 20), is less dispersed in Asia, such as in Korea (39) and Japan (32), and causes gastroenteritis and occasional outbreaks in human and wild or domestic animals. Type ACSSuT \textit{S. enterica} serovar Typhimurium DT104 isolates not only have been frequently found in human (13) but have been commonly found in geese in the present study (Table 2). In Asia, several phage types of \textit{S. enterica} serovar Typhimurium DT104 have been isolated from poultry and animals, such as phage types 13, 120, and DT104 in chickens (19) and phage type 90 in humans and animals (36). In this study, diverse phage types were found mainly in type ACSSuT \textit{S. enterica} serovar Typhimurium isolates belonging to genotypes I and II, and some phage types, types 8, 12, and U283, were found only in geese (Fig. 2), suggesting that geese are a reservoir of diverse multidrug-resistant \textit{S. enterica} serovar Typhimurium isolates.

**TABLE 3. Genotype distribution of 58 \textit{S. enterica} serovar Typhimurium strains from different farms**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of isolates from:</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-FAN</td>
<td>B-CBC</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Ib</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**FIG. 2. Dendrogram and PFGE patterns of XbaI-digested genomic DNA of \textit{S. enterica} serovar Typhimurium isolates.** The dendrogram was constructed based on the unweighted-pair group method using average linkages algorithm and the Dice similarity coefficient by using BioNumerics software with 3% optimization and 1% position tolerance. The genotypes are indicated as I, II, III, and IV. \textit{S. enterica} serovar Braendrup strain H9812 was the PFGE size marker.
REFERENCES


31. Reference deleted.
