**Original Article**

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# Phenotypic and genotypic antimicrobial resistance and virulence genes of *Salmonella enterica* isolated from pet dogs and cats

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*Salmonella enterica* isolates (n = 122), including 32 serotypes from 113 dogs and 9 cats, were obtained from household dogs (n = 250) and cats (n = 50) during 2012–2015. The isolates were characterized by serotyping, antimicrobial resistance phenotyping and genotyping, and virulence gene screening. Serovars Weltevreden (15.6%) and Typhimurium (13.9%) were the most common. The majority (43%) of the isolates were multidrug resistant. The dog isolates (12.3%) harbored class 1 integrons, of which the *dfrA12-aadA2* cassette was most frequent (66.7%). The only class integron in serovar Albany was located on a conjugative plasmid. Two ESBL-producing isolates (*i.e.*, a serovar Krefeld and a serovar Enteritridis) carried $bla_{TEM}$ and $bla_{CTX-M}$, and the $bla_{TEM}$ gene in both was horizontally transferred. Of the plasmid-mediated quinolone resistance genes tested, only *qnrS* (4.9%) was detected. Most *Salmonella* isolates harbored *invA* (100%), *prgH* (91.8%), and *sipB* (91%). Positive associations between resistance and virulence genes were observed for $bla_{PSE-1}$/*orgA*, *cmlA*/*spaN*, *tolC*, and *sul1*/*tolC* ($p < 0.05$). The results suggest that companion dogs and cats are potential sources of *S. enterica* strains that carry resistance and virulence genes and that antimicrobial use in companion animals may select for the examined *Salmonella* virulence factors.

**Keywords:** *Salmonella enterica*, antimicrobial resistance, integrons, pets, virulence factors

## Introduction

Strains of *Salmonella* (*S.*) *enterica* that are resistant to a wide range of antimicrobial drugs have emerged and are now a serious public health issue worldwide. Food animals have been decried as major reservoirs for the transmission of multidrug resistant (MDR) *Salmonella* to humans and also for their part in the dissemination of resistance genes [31]. In previous studies, companion animals have been suggested as potential sources of antimicrobial-resistant *Salmonella* [26,33]; however, less attention has been given to their contribution to the spread of antimicrobial resistance (AMR) than that given to food animals.

In small animal practice, antimicrobial agents, including those licensed for human use and of primary importance in the treatment of human infections, are frequently used for infection treatment [17]. Bacterial culture and antimicrobial susceptibility testing are not always performed when making antimicrobial choices, leading to inappropriate empirical treatment. Veterinarians may prescribe broad-spectrum or newer generation antibiotics as first-line treatment drugs to ensure successful antimicrobial therapy. Such imprudent antimicrobial usage, similar to that observed in humans, could create selective pressure for AMR bacteria, including MDR *Salmonella* in companion animals. In modern society, the relationship between humans and their pet animals has drastically changed, and companion dogs and cats are usually kept inside the household. In such instances, close physical contact between pet animals and humans favors pet-to-human transmission of bacteria either by direct contact or through the domestic environment; moreover, children have the potential to be at higher risk than adults [17].

Currently, it is difficult to assess the impact of small animal-related AMR on public health due to limited availability

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of data. AMR in pet animals is not systematically monitored in most countries, and existing data is quite scattered. In addition, descriptions of linkages between resistance and virulence genes in *Salmonella* isolates from pet animals have not been reported. These knowledge gaps indicate the need for more studies into AMR in small animals. Therefore, in this study, we characterized phenotypic and genotypic characteristics of AMR and the prevalence of virulence factors in *S. enterica* isolated from pet dogs and cats.

## Materials and Methods

### *Salmonella* isolates

*S. enterica* (n = 122) isolated from household dogs (n = 113) and cats (n = 9) were obtained from the bacterial collection of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand. Khon Kaen is located in northeast Thailand, the most populous region of the country. All *S. enterica* strains were isolated from fecal samples obtained by rectal swabs from household dogs (n = 250) and cats (n = 50) presented for treatment at Khon Kaen Animal Hospital, Khon Kaen University, or four local veterinary clinics in Khon Kaen province during 2012–2015. The animals exhibited a variety of health conditions, but respiratory infection, parasitic infestation, heartworm disease, skin disorder, and diarrhea formed the majority of health conditions. The clinical symptoms of each animal were not available for inclusion in this study. Moreover, there were no exclusion criteria for the dogs or cats recruited, and details on their antibiotic treatment protocols were not revealed. *Salmonella* isolation was performed by using a standard method [22]. Briefly, each rectal swab was placed in 250 mL buffer peptone water (Merck, Germany) and incubated at 37°C overnight. The culture suspension was then spotted on modified semisolid Rappaport medium (Merck) and incubated at 42°C for 24 h. Next, the *Salmonella* isolates were isolated on xylose-lysine-desoxycholate agar (Merck) and Hektoen enteric agar (Merck) at 37°C for 24 h. Typical colonies of *Salmonella* were biochemically confirmed by using triple sugar iron agar (Merck) and motility indole-lysine agar (Merck). All isolates were serotyped by using a slide agglutination test at the Center of Antimicrobial Resistance Monitoring in Foodborne Pathogens (in cooperation with World Health Organization), Faculty of Veterinary Science, Chulalongkorn University, Thailand. One colony of each serotype in each positive sample was collected and stored in 20% glycerol at −80°C.

### Antimicrobial susceptibility testing and screening of extended-spectrum-β-lactamases

All strains were tested for minimum inhibitory concentrations (MICs) by using the two-fold agar dilution technique described previously [11] (concentration breakpoints indicated in parentheses): ampicillin (32 μg/mL), ceftriaxone (64 μg/mL), chloramphenicol (32 μg/mL), gentamicin (8 μg/mL), ciprofloxacin (4 μg/mL), spectinomycin (128 μg/mL), streptomycin (32 μg/mL), sulfamethoxazole (512 μg/mL), tetracycline (16 μg/mL), and trimethoprim (16 μg/mL). Briefly, bacterial isolates were grown on Muller-Hinton agar (MHA; BD Diagnostic Systems, USA) at 37°C overnight. Subsequently, a bacterial suspension with turbidity equivalent to 0.5 McFarland was prepared in a 0.85% NaCl solution and then diluted 10-fold. The standardized suspension was inoculated by using a multipoint inoculator onto MHA containing different concentrations of antibiotics. The inoculated MHA plates were incubated at 37°C for 16 to 18 h.

For initial screening of extended-spectrum-β-lactamase (ESBL) production, a disk diffusion method using cefotaxime (30 μg), cefpodoxime (10 μg) and ceftazidime (30 μg) was used [11]. Briefly, a standardized inoculum of the test isolate was applied on MHA plates. The antimicrobial disks were placed on the inoculated agar surface and the plates were incubated at 37°C for 16 to 18 h. Those isolates yielding an inhibition clear zone of at least 11 mm diameter were defined as resistant strains. The isolates showing resistance to at least one cephalosporin test were then subjected to ESBL-phenotypic confirmation by using a combination disk assay [11] using cefotaxime/cefotaxime (30 μg) + clavulanic acid (10 μg) and ceftazidime/ceftazidime (30 μg) + clavulanic acid (10 μg) (Oxoid, England). The isolates demonstrating an increase of $\geq$ 5 mm in the zone of inhibition in a combination disk were considered ESBL positive. The strains exhibiting concurrent resistance to three or more different classes of antimicrobials were considered MDR [38]. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as quality control bacteria.

### DNA preparation, PCR amplification, and DNA sequencing

Total bacterial DNA of each isolate was prepared by boiling a bacterial pellet in sterile distilled water for 10 min [27], which was then used as template DNA in all PCR reactions, except those for amplifying the quinolone resistance-determining regions (QRDRs) in *gyrA*, *gyrB*, *parC*, and *parE*. The QRDR amplification used chromosomal DNA that was extracted by using a QIAamp DNA mini kit (Qiagen, Germany). All PCR amplifications were performed by using Readymix PCR MasterMix (Sigma-Aldrich, USA) according to the manufacturer's instructions. The PCR amplification products were purified by using Nucleospin extract II (Macherey-Nagel, Germany) and the nucleotide sequences were determined at Macrogen (Korea). The DNA sequences were compared for homology with the GenBank sequence database by using the BLAST program (National Center for Biotechnology Information, USA).

### Detection of class 1 integrons

Class 1 integrons were detected by performing PCR of *intI1* [9,13]. The presence of inserted-gene cassettes in the *intI1*-positive isolates were determined by PCR assays using primers specific for the conserved region (5'-3' CS-PCR) and DNA sequencing [27]. The PCR amplicons were collected from gel, purified, and submitted for DNA sequencing.

### Detection of resistance genes, virulence genes, and mutation in QRDR

The isolates were grouped on the basis of resistance phenotype and determined for the presence of corresponding resistance genes: $bla_{PSE-1}$, and $bla_{TEM}$ encoding β-lactam resistance (n = 62); *catA*, *catB*, and *cmlA* encoding chloramphenicol resistance (n = 28); *aadB* encoding gentamicin resistance (n=9); *tetA* and *tetB* encoding tetracycline resistance (n = 47); *dfrA1*, *dfrA10*, and *dfrA12* trimethoprim resistance (n = 39); *aadA1* and *aadA2* encoding spectinomycin and streptomycin resistance (n = 20); *strA* and *strB* encoding streptomycin resistance (n = 32); *sul1*, *sul2*, and *sul3* encoding sulphonamide resistance (n = 122) [9,10]. The $bla_{CTX-M}$, $bla_{SHV}$, $bla_{CMY-1}$, and $bla_{CMY-2}$ were additionally examined in the ESBL-positive strains (n = 2). All *Salmonella* isolates were screened for plasmid-mediated low-fluoroquinolone resistance (PMQR) genes including *qnrA*, *qnrB*, *qnrS* [41], *qepA* [48], and *aac(6')-lb-cr* [32].

The regions covering the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* of the ciprofloxacin-resistant isolate (n = 1) were amplified and subjected to DNA sequencing [10]. The QRDRs of *gyrA* and *parC* obtained from a ciprofloxacin-susceptible isolate were included as controls.

The presence of virulence genes associated with the pathogenesis of *Salmonella*, including *invA*, *pefA*, *sipB*, *spvC*, *prgH*, *spaN*, *orgA*, *tolC*, and *rck* genes, was examined in all *Salmonella* isolates [6,40].

### Conjugation studies

The *Salmonella* isolates carrying class 1 integrons with resistance gene cassettes (n = 5) and those with ESBL genes (n = 2) were used as donors in our conjugation experiments [5]. *E. coli* K12Rif$^r$, a rifampicin-resistant derivative of *E. coli* K12 strain MG1655 (MIC = 256 µg/mL), was used as the recipient. Transconjugants were selected on Luria-Bertani (LB) agar (Difco, BD Diagnostic Systems, USA) containing rifampicin (32 µg/mL) and one of the following antibiotics: ampicillin (100 µg/mL), cefoperazone (2 µg/mL), trimethoprim (10 µg/mL), or streptomycin (50 µg/mL) and were confirmed on eosin methylene blue agar (EMB; Difco, USA). Transfer of class 1 integrons was confirmed by performing PCR as described above.

### Statistical analysis

Pearson's chi-squared test was performed for the analysis of associations between resistance and virulence genes by using SPSS (ver. 17.0, SPSS, USA). Associations among genes were considered significant when $p$ values were $< 0.05$. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by using the same software [16,37]. An OR $>$ 1 indicated a positive association while an OR $<$ 1 represented a negative association.

## Results

### *Salmonella* serotypes

Among the 122 *Salmonella* isolates, 33 serovars were identified.

**Table 1.** *Salmonella* serotypes isolated from dogs and cats (n = 122)

| Serotype | Number (%) |
|---|---|
| Aberdeen | 2 (1.6%) |
| Agona | 1 (0.8%) |
| Albany | 1 (0.8%) |
| Anatum | 1 (0.8%) |
| Bovismorbificans | 2 (1.6%) |
| Brunei | 5 (4.1%) |
| Eastbourne | 3 (2.5%)* |
| Enteritidis | 2 (1.6%) |
| Emek | 2 (1.6%) |
| Give | 6 (4.9%) |
| Hadar | 1 (0.8%) |
| Havana | 1 (0.8%) |
| Hvittingfoss | 5 (4.1%)† |
| Kedougou | 4 (3.3%) |
| Krefeld | 1 (0.8%) |
| Lexington | 1 (0.8%) |
| Mgulani | 1 (0.8%) |
| Montevideo | 1 (0.8%) |
| Muenster | 1 (0.8%) |
| Panama | 2 (1.6%) |
| Schwarzengrund | 2 (1.6%) |
| ser. 4,[5],12:i:- | 7 (5.7%) |
| ser. 8,20:-:- | 1 (0.8%) |
| ser. 9,12:-1,5 | 1 (0.8%) |
| Rissen | 10 (8.2%) |
| Stanley | 11 (9.0%) |
| Typhimurium | 17 (13.9%)† |
| Virchow | 7 (5.7%)† |
| Urbana | 1 (0.8%) |
| Wandsworth | 1 (0.8%) |
| Wentworth | 1 (0.8%) |
| Weltevreden | 19 (15.6%)‡ |
| Worthington | 1 (0.8%) |
| Total | 122 |

*Two isolates were from cats. †One isolate was from cats. ‡Four isolates were from cats.

Serovar Weltevreden was predominant (15.6%) followed by serovars Typhimurium (13.9%), Stanley (9.01%), and Rissen (9.01%) (Table 1). Serovar Weltevreden was most common among the isolates from both dogs (n = 15) and cats (n = 4).

### Antimicrobial resistance profile

Resistance to at least one antimicrobial agent was observed in all *Salmonella* isolates and 43% were MDR (Fig. 1). All isolates were resistant to sulfamethoxazole (100%), followed by resistance to ampicillin (50.8%) and tetracycline (32%). Resistance rates to ceftriaxone (2.5%) and ciprofloxacin (0.8%) were notably low. Only two dog isolates (*i.e.*, a serovar Krefeld and a serovar Enteritidis) were resistant to cephalosporins, including cefotaxime (1.6%) and cefpodoxime (1.6%), and were confirmed to be ESBL-positive strains. Among the cat isolates, a serovar Typhimurium isolate was resistant to multiple drugs (*i.e.*, ampicillin, streptomycin, sulfamethoxazole, and tetracycline) while the others were resistant to sulfamethoxazole only.

### Characteristics and transfer of class 1 integrons

Fifteen (12.3%) isolates from dogs carried *intI1*, but no *intI1* was detected in the cat isolates. Analysis of the gene cassette arrays revealed that five *intI1*-positive isolates yielded cassette PCR amplicons of variable region of 2500 bp or 1300 bp. The others carried the 150 bp variable region of empty integrons. Two complete gene cassettes, which encoded dihydrofolate reductase responsible for trimethoprim resistance (*dfrA12*) and aminoglycoside adenyl transferase responsible for streptomycin-spectinomycin resistance (*aadA2*) were identified. Four Rissen serovar isolates carried class 1 integrons with the 2500 bp/*dfrA12-aadA2* variable region. A serovar Albany isolate harbored class 1 integron with the 1300/*dfrA12* variable region. Only class 1 integrons with the *dfrA12* gene cassette in the serovar Albany was horizontally transferred to the *E. coli* recipient.

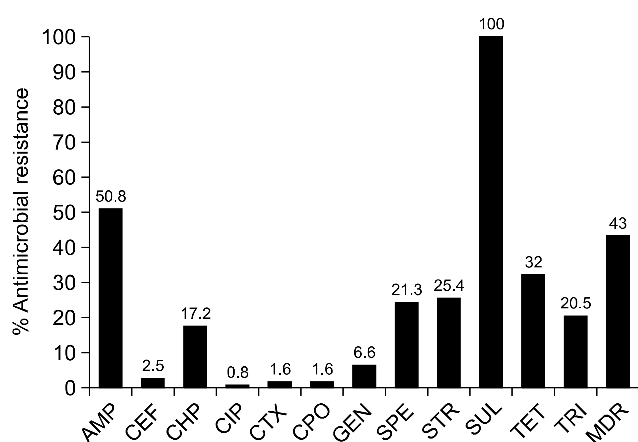


**Fig. 1.** Distribution of antimicrobial resistance in *Salmonella* serovars isolated from dogs and cats (n = 122). AMP, ampicillin; CEF, ceftriaxone; CHP, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CPO, cefpodoxime; GEN, gentamicin; SPE, spectinomycin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; TRI, trimethoprim; MDR, multidrug resistance.

### Non-class 1 integron-borne resistance and ESBL genes

The resistance genes commonly detected were those encoding resistance to chloramphenicol (*cmlA*, 60.1%), streptomycin (*strA-strB*, 59.4%), gentamicin (*aadA1*, 55.6%), and tetracycline (*tetA* and *tetB*, 51.1% of each) (Table 2). Twenty-two isolates (18%) carried at least two genes encoding identical resistance phenotypes. The MDR serovar Typhimurium from cats carried multiple genes including $bla_{TEM}$, *tetB*, and *strA-strB*. Up to 54% of the isolates did not carry any of the resistance-encoding

**Table 2.** Distribution of antimicrobial resistance-encoding genes among the *Salmonella* isolates from dogs and cats (n = 122)

| Resistant isolate (n) | Resistance gene | N (%) |
|---|---|---|
| Ampicillin (62) | $bla_{PSE1}$ | 2 (3.2) |
| | $bla_{TEM}$ | 25 (40.0) |
| | both | 1 (1.6) |
| Chloramphenicol (28) | *catA* | 4 (14.3) |
| | *cmlA* | 17 (60.7) |
| | both | 1 (3.6) |
| Gentamicin (9) | *aadB* | 1 (11.1) |
| Streptomycin (32) | *aadA1* | 4 (12.5) |
| | *aadA2* | 1 (3.1) |
| | *strA-strB* | 19 (59.4) |
| | *aadA1*, *aadA2* | 1 (3.1) |
| | *aadA1*, *strA-strB* | 1 (3.1) |
| | *aadA1*, *aadA2*, *strA-strB* | 1 (3.1) |
| Spectinomycin (20) | *aadA1* | 16 (80.0) |
| | *aadA2* | 2 (10.0) |
| | both | 2 (10.0) |
| Sulfamethoxazole (122) | *sul1* | 17 (13.9) |
| | *sul2* | 1 (0.8) |
| | *sul3* | 10 (8.2) |
| | *sul1*, *sul2* | 1 (0.8) |
| | *sul1*, *sul3* | 2 (1.6) |
| Tetracycline (47) | *tetA* | 24 (51.1) |
| | *tetB* | 24 (51.1) |
| | both | 10 (21.3) |
| Trimethoprim (39) | *dfrA1* | 2 (5.13) |
| | *dfrA10* | 10 (25.6) |
| | *dfrA12* | 3 (7.7) |
| | *dfrA1*, *dfrA12* | 2 (5.1) |
| Quninolones (122) | *qnrS* | 6 (4.9) |
| | *qnrA* | 0 |
| | *qnrB* | 0 |
| | *qepA* | 0 |
| | *aac(6')-lb-cr* | 0 |

genes tested (data not shown). Both ESBL-producing isolates carried $bla_{TEM}$ and $bla_{CTX-M}$, of which only $bla_{TEM}$ was horizontally transferred in the conjugation experiments.

In addition, associations among the resistance genes were analyzed (Table 3). Only positive associations were observed ($p < 0.05$), of which the strongest positive association was between *sul3* and $bla_{TEM}$.

### The PMQR genes and mutations within the QRDRs of *gyrA* and *parC*

The only ciprofloxacin-resistant isolate was a serovar Stanley (CIP MIC = 16 μg/mL). This isolate did not carry any mutations in *gyrA*, *gyrB*, *parC* and *parE*. Of the PMQR genes tested, only *qnrS* was identified in 6 *Salmonella* isolates from dogs (4.9%): 3 serovar Give (CIP MIC = 2 μg/mL) isolates and one isolate each of serovar Emek (CIP MIC = 1 μg/mL), serovar Enteritridis (CIP MIC = 2 μg/mL), and serovar Krefeld (CIP MIC = 2 μg/mL).

### Occurrence of virulence genes

All *Salmonella* isolates from dogs and cats harbored *invA* (100%), and most isolates carried *prgH* (91.8%) and *sipB* (91%). The other virulence genes were present to lesser extents including *tolC* (85.2%), *spaN* (31.1%), *orgA* (5.74%), and *rck* (0.8%). The latter was detected in one serovar Enteritidis isolate from a dog. None of the isolates were positive to *spvC* and *pefA*.

Positive associations ($p < 0.05$) were observed between the following virulence gene pairs: *sipB*/*prgH* (OR 145.33, 95% CI 21.14–999.13) and *spaN*/*tolC* (OR 34.44, 9% CI 1.46–8.13). In addition, positive correlations ($p < 0.05$) were found between resistance genes and virulence genes including $bla_{PSE1}$/*orgA*; *cmlA*/*spaN*, *tolC*, and *sul1*/*tolC* (Table 4).

## Discussion

This study demonstrated the wide distribution of MDR *Salmonella* with virulence genes among pet dogs and cats, as well as the occurrence of significant correlations between the presence of resistance genes and virulence genes. However, because the isolates were not randomly collected, the results of this study do not necessarily represent the characteristics of other regions of Thailand. Despite that limitation, the data presented in this study can be used in comparisons with those in reports from other regions and on other animals. However, there are only a few reports on the study of AMR phenotypes and genotypes in companion animals.

The predominant serovars in this collection of *Salmonella* strains were Weltreveden and Typhimurium, which are also the most common serovars implicated in human salmonellosis in

**Table 3.** Associations between resistance genes of *Salmonella enterica* isolated from dogs and cats (n = 122)

| | Associations of gene*, odds ratio (95% confidence interval)† | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $bla_{PSE1}$ | *tetB* | *cmlA* | *strB* | *sul1* | *sul3* | *dfrA10* | *dfrA12* | *aadA1* |
| $bla_{PSE1}$ | NA | NA | – | NA | NA | – | NA | NA | – |
| $bla_{TEM}$ | NA | 5.08 (1.90–13.56) | 4.60 (1.56–13.61) | – | 4.60 (1.56–13.61) | 54 (6.40–455.63) | 4.6 (1.22–17.40) | – | 14.46 (4.37–47.873) |
| *tetA* | NA | 11 (3.62-33.43) | – | 13.19 (4.16-41.84) | – | NA | 7.62 (1.94–29.90) | NA | – |
| *tetB* | NA | NA | – | 26.04 (7.75–87.47) | 12.97 (4.10–41.05) | – | – | NA | – |
| *cmlA* | – | NA | NA | – | – | – | 5.08 (1.26–20.40) | – | 5.18 (1.58–17.02) |
| *catA* | NA | NA | NA | NA | – | NA | NA | 19.33 (1.00 -276.41) | NA |
| *strB* | NA | 26.04 (7.75–87.47) | – | NA | 7.60 (2.43–23.73) | – | – | – | NA |
| *sul1* | NA | 12.97 (4.10–41.05) | – | NA | NA | – | – | NA | NA |
| *sul3* | – | – | – | – | – | NA | 11.78 (2.60–53.26) | NA | 15.30 (3.69–63.00) |
| *dfrA10* | NA | – | 5.077 (1.26-20.40) | – | – | 11.78 (2.60-53.26) | NA | NA | 26.70 (5.87–121.41) |

–, no significant association ($p \geq 0.05$); NA, indicates no result available (or result could not be calculated because none of the isolates carried one of the combinations of virulence genes and resistance genes or the value of one the genes was a constant or zero). *Only genes with a significant association ($p < 0.05$) with virulence genes are shown. †Odds ratio for significant associations between genes (95% confidence interval in parenthesis).

**Table 4.** Associations between resistance and virulence genes among *Salmonella enterica* isolated from dogs and cats (n = 122)

| Virulence gene | Associations of gene*, odds ratio (95% confidence interval)† | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $bla_{PSE1}$ | *tetB* | *cmlA* | *strB* | *sul1* | *sul3* | *dfrA10* | *dfrA12* | *aadA1* |
| *spaN* | – | – | 3.93 (1.36–11.32) | – | – | – | – | NA | – |
| *orgA* | 19 (1.06–342.15) | – | – | – | – | NA | NA | NA | NA |
| *tolC* | – | – | 3.78 (1.03–13.94) | – | 3.78 (1.03–13.94) | – | – | NA | – |

–, no significant association ($p \geq 0.05$); NA, no result available (or result could not be calculated because none of the isolates carried one of the combinations of virulence genes and resistance genes or the value of one of the genes was a constant or zero. *Only genes with a significant association ($p < 0.05$) with virulence genes are shown. †Odds ratio for significant associations between genes (95% confidence interval in parenthesis).

many world regions [12,15]. Serovar Weltreveden has been frequently isolated from many sources including raw vegetables [43], seafood [19], as well as duck and water [1]. It has been suggested that water-related sources are the most-likely reservoirs for the widespread occurrences of serovar Weltreveden [19]. In addition, humans and dogs share exposure to common sources of potential *Salmonella* infection [45].

There was a high prevalence of *Salmonella* isolates that were resistant to multiple drugs (43%), including common antimicrobials (*e.g.*, sulfamethoxazole, ampicillin, and tetracycline), a result that supports those in previous studies from different countries [23,45]. This is not surprising because these antimicrobials have been widely used for infection treatment in dogs and cats. The sulfamethoxazole resistance rate in this study was much higher than that in a previous report on household and stray dogs in northern Taiwan [45]. In contrast, the streptomycin resistance rate in our study (25.4%) was much lower than those reported in previous studies in Trinidad [39], Ontario [26], and northern Taiwan [45]. In addition, the prevalence of ciprofloxacin resistance in our study was very limited (0.8%), which is similar to previous studies in which no ciprofloxacin resistance was reported [26,45]. Taken together, these observations indicate that AMR prevalence in the *Salmonella* isolates from dogs varies by geographical region and reflects different antimicrobial use in pet animals in different countries. It should be noted that the results may be, at least partly, affected by sample size and different antimicrobial susceptibility testing methods used in different laboratories.

When compared to livestock, resistance rates observed in this study were generally higher than those in healthy dairy cows [8]. This is likely due to more frequent use of antimicrobial in pets. In contrast, resistance rates of most antimicrobials (*e.g.*, chloramphenicol, gentamicin, streptomycin, tetracycline, and trimethoprim), except sulphamethoxazole, were lower than those in broilers and pigs [10,24]. The apparent inconsistencies are likely attributable to different antimicrobial types and applications in different animal species.

Class 1 integron-mediated antimicrobial resistance is common among diverse *Salmonella* serovars and has been extensively studied in livestock and foods of animal origin. To our knowledge, the present study is the first to describe class 1 integrons in *Salmonella* isolates from household dogs and cats. The most prevalent resistance gene cassette was *dfrA12-aadA2*, which has been widely observed to be associated with class 1 integrons among *Salmonella* isolates from food animals [21,28] and was the most frequently occurring resistance gene cassette identified in isolates from pigs, broilers, dairy cow, pork, and chicken meat in Thailand [8,24,47]. In addition, the resistance genes observed in the present study were previously observed in *Salmonella* from humans, food animals, and food products [10]. The prevalence of some resistance genes (*i.e.*, $bla_{TEM}$, *aadB*, *tetA*, *sul1*, and *sul3*) was similar to the relatively high rate detected in broiler and pig isolates [10]. These results indicate the breadth of the circulation and exchange of resistance determinants among human, food animals, and pet animals. This is supported by the observation of horizontal transfer of class 1 integrons with the *dfrA12* gene cassette in this study. However, it is difficult to ascertain the direction of the actual gene transfer [17].

Twenty-six percent of the *Salmonella* isolates in this study contained multiple genes specifying identical resistance phenotypes, which is similar to results in a previous study of diarrheic dogs [46]. It is possible that a resistance gene is chromosomally located while others are on plasmids. In addition, strong associations ($p < 0.05$) were observed for certain resistance genes pairs (*e.g.*, $bla_{TEM}$/*sul3*; *dfrA10*/*aadA1*, and *tetB*/*strB*), suggesting their colocalization on the same genetic elements (*e.g.*, plasmids, transposons) and the possible co-selection of many resistance genes by a single antibiotic. However, the resistance-encoding genes tested were not found in many isolates (n = 66, 54%), indicating existence of other resistance mechanisms.

ESBL-producing *Salmonella* is a serious concern because cephalosporins are drugs of choice for Salmonellosis treatment in children [18]. In this study, a low incidence of ESBL-producing *Salmonella* was observed. This was likely because, due to their high price, use of cephalosporins is less common in companion animals in this region. Despite the limited occurrence, ESBL-producing *Salmonella* carrying $bla_{TEM}$ and $bla_{CTX\text{-}M}$ can have important implications with regard to the spread of ESBL genes. Unlike infectious diseases, AMR determinants can be transmitted from one bacterial host to another, even among low numbers of bacteria [17]. The latter was supported by the observation of $bla_{TEM}$ located on a conjugative plasmid in this study.

Since PMQR genes confer only low-level quinolone resistance [42], the PMQR genes were screened in all *Salmonella* isolates. Only *qnrS* was detected, and at a low rate (4.9%), in the ciprofloxacin-susceptible isolates. This is clinically important because a low prevalence of PMQR genes can facilitate the selection of higher-level resistance mutations and the ciprofloxacin-susceptible isolates may readily develop resistance to fluoroquinolones [35].

It is of particular interest to note that a serovar Stanley isolate lacking mutation in *gyrA*, *gyrB*, *parCi*, and *parE* exhibited high-level ciprofloxacin resistance (16 μg/mL). This is in agreement with a previous study that reported the presence of *Salmonella* isolates harboring no mutations in both target genes and having a ciprofloxacin MIC comparable to that for the isolate in this study (8–16 μg/mL) [29]. The absence of mutations in *gyrB* and *parE* is not surprising because *gyrB* and *parE* mutation are rare in *Salmonella*, other than in serovar Typhimurium [30]. The results indicate the existence of uncharacterized mechanisms underlying ciprofloxacin resistance in this isolate. In general, the fluoroquinolone resistance phenotype can be associated with several mechanisms such as altered cell permeability, enzymatic inactivation, expression of an active drug efflux, and competitive inhibition of drug binding [36]. A previous study demonstrated that ciprofloxacin-resistant isolates with no *gyrA* and *parC* mutations had a reduced accumulation of ciprofloxacin, compared with that in the susceptible strains, and additionally, they were cross-resistant to other antibiotics in different classes [34]. Those authors suggested that drug efflux moderated the ciprofloxacin accumulation. The AcrAB-TolC efflux pump is a major contributor to multidrug resistance in Gram-negative bacteria and inactivation of the *acrAR* operon and/or *tolC* could lead to increased fluoroquinolone susceptibility [4]. Another study revealed alteration or disappearance of outer membrane proteins (*i.e.*, OmpA, OpmC, OmpD, and OmpF) in quinolone resistant *Salmonella* strains [36]. However, the mechanisms responsible for the high ciprofloxacin MIC were not described in that study.

In general, AMR is more common in pathogenic bacteria due to the more frequent and intense exposure to antimicrobial agents of pathogenic strains [3], and the associations between AMR and virulence traits has been reviewed previously [2]. The high prevalence of *invA*, *prgH*, and *sipB* observed in this study (91–100%) is in agreement with those in previous studies of various *Salmonella* sources, *i.e.*, pork, swine, and dogs [44,47] and indicates that pet dogs and cats serve as reservoirs of invasive *Salmonella*. Interestingly, 14.8% of the isolates lacked *tolC* encoding for an outer membrane protein with a common function in protein export and multidrug efflux [49]. These isolates were resistant only to sulfamethoxazole (data not shown). This is an uncommon observation and differed from those in previous studies [47]. Further investigations are warranted to elucidate the reason underlying the absence of *tolC* in these strains. The *rck* gene has been identified in virulence plasmids of serovar Typhimurium and serovar Enteritidis [40]. In the present study, only serovar Enteritidis isolate was positive to *rck*. In addition, the presence of some resistance genes was significantly associated with increased prevalence of certain virulence genes (*i.e.*, $bla_{PSE1}$/*orgA*; *cmlA*/*span*; *cmlA*/*tolC* and *sul1*/*tolC*), suggesting that antimicrobial use may co-select for virulence genes, and a possible linkage of virulence and resistance determinants on genetic elements such as plasmids and transposons may exist [25]. It is possible that both resistance and virulence genes are located on the same plasmid or on different but compatible plasmids. Plasmid analysis will help in elucidating the spread of virulence genotypes and antibiotic resistance; however, such investigation was not pursued in this study. Co-existence of resistance and virulence genes on a large drug resistance/virulence plasmid, pSCV, was previously characterized in serovar Choleraesuis [7], and it was suggested that the resistance genes (*i.e.*, *sul1* and $bla_{TEM\text{-}1}$) were likely acquired through recombination. A virulence/resistance hybrid plasmid, pUO-StVR2, carrying a complex resistance island and several virulence genes was demonstrated in serovar Typhimurium [20]. Taken together, these results indicate the presence of a connection between resistance and virulence genes in *Salmonella*. Further, the positive associations between resistance and virulence genes observed in this study suggest that antimicrobial use in household dogs and cats may generate selective pressure for co-selection of resistance and virulence determinants, which are or can become beneficial to the spread and persistence of both types of genes among the *Salmonella* population [14].

In conclusion, the results in this study highlight the role of household dogs and cats as potential sources of AMR *Salmonella* strains with a wide range of AMR determinants and virulence genes; strains that may spread to humans and into the environment. The results should be used to emphasize the responsibility of veterinarians and relevant authorities to develop prudent-use guidelines to minimize the emergence and spread of AMR in bacterial strains originating from companion

animals. The collection of reliable data on the frequency of AMR occurrence is a prerequisite to developing guidelines and regulations on the prudent use of antimicrobial agents. To date, such activity related to companion animals has been limited. Therefore, it is strongly recommended that AMR monitoring and surveillance programs should include bacteria originating from dogs, cats, and other pet animals.

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## Conflict of Interest

The author declare no conflict of interests.

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