Full Length Research Paper

Salmonella genomic island 1 and class 1 integron in Salmonella isolates from stray dogs

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Stray dogs may be asymptomatic carriers of Salmonella. We identified 160 Salmonella isolates, representing 28 different serovars, from stray dogs of central Taiwan. A total of 42 (27%) of the isolates (15 different serovars) were positive for class 1 integrons, with four kinds of gene cassettes. SGI1 were present in 22 of the class 1 integron-positive isolates. This study indicates that widespread occurrence of multidrug resistant Salmonella in stray dogs and the diversity of class 1 integrons and SGI1 in different Salmonella serovars. Stray dogs may play an important role as carriers of multidrug resistant Salmonella.

Key word: Class 1 integrons, Salmonella enterica, stray dogs.

INTRODUCTION

Zoonotic transmission of Salmonella spp. can occur through direct exposure to the feces of animals. In particular, dogs are a possible source of shedding Salmonella for dog owners and their communities (Sanchez et al., 2002). Dogs are often asymptomatic carriers of Salmonella, and may shed the organism even though they exhibit no signs of illness. Stray dogs often live close to communities, so the incidence of Salmonella infection in stray dogs is an important public health concern (Butcher, 1999). In the United States, Wright et al. (2005) have suggested the potential for zoonotic transmission of multidrug-resistant Salmonella from stray dogs to humans and that environmental contamination may serve as an ongoing source of human infection. A previous study showed that the prevalence of Salmonella in the feces of stray dogs of Turkey was between 0.0 and 23.5% (Kocabiyik et al., 2006). The prevalence of Salmonella in stray dogs of northern Taiwan was reported to be 6.3%, with some isolates having multidrug resistance (Tsai et al., 2007). Multidrug resistance is associated with the presence of mobile genetic elements that enable horizontal and vertical transfer of antibiotic resistance genes in Gram-negative bacteria (van Essen-Zandbergen et al., 2007). In particular, integrons are genetic elements that can capture gene cassettes from the environment and incorporate them into bacterial genomes by the use of site-specific recombination. Four classes of integrons are associated with drug-resistant gene cassettes. Class 1 integrons are the most common...
type in multidrug-resistant Salmonella (Fluit, 2005).

The 43-kb Salmonella genomic island 1 (SGI1) is a Salmonella-derived integrative mobile element that was originally identified in an epidemic of multidrug-resistant Salmonella Typhimurium DT104 (Boyd et al., 2001). SGI1 contains a multidrug resistance region, with numerous antibiotic resistance genes. Since the identification of SGI1, variants of the SGI1 region have been described in a wide variety of Salmonella enterica serovars (Doublet et al., 2009). These SGI1 variants may have been generated by homologous recombination or resistance gene cassette exchange.

Most studies of the prevalence and other characteristics of antimicrobial resistance genes and integrons in Salmonella have focused on strains from clinical and veterinary sources. However, very little is known about the transmission of integrons from Salmonella that have been isolated from stray dogs. In this study, we performed rectal swabs from stray dogs of different shelters in central Taiwan and cultured these swabs for Salmonella. Then, we determined the serotype, antimicrobial drug resistance characteristics of the Salmonella isolates.

MATERIALS AND METHODS

Sample isolates

Between November 2005 and March 2007, we collected rectal swabs of 1002 stray dogs from 5 animal shelters in Taichung County (483 dogs), Taichung City (301 dogs), Changhua County (125 dogs), Nantou County (52 dogs) and Miaoli (41 dogs). Each dog was sampled only once for testing. All dogs were cross breeds and age between 2 months and 5 years. A total of 554 (55.3%) were female and 448 dogs (44.7%) were male. Each faecal sample was homogenized in 10 times volume of buffered peptone water (BPW) and incubated at 37°C for 18 to 24 h. All BPW-enriched samples enrichment in Rappaport Vassiliadis (RV) and Modified Semi-Solid Rappaport Vassiliadis (MSRV) broth 24 h. After enrichment, samples were plated onto two selective media: xylose lysine deoxycholate (XLD) agar and bismuth-sulfite (BS) agar. Presumptive Salmonella colonies were selected and subjected to biochemical tests. Salmonella serotypes were determined by slide agglutination with commercial antisera (S & A Reagents Lab, Bangkok, Thailand and Denka Seiken, Tokyo, Japan) following the Kauffman–White scheme (Rowe and Hall, 1989; Popoff, 2001).

Antimicrobial susceptibility testing

The sensitivity of Salmonella isolates to amoxycillin/clavulanic acid (20/10 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), gentamicin (10 µg), nalidixic acid (30 µg), ceftriaxone (30 µg), tetracycline (30 µg), florenicil (30 µg) and trimethoprim/sulphamethoxazole (1.25/23.75 µg) was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, with Escherichia coli (ATCC 25922) used as a control (Bauer et al., 1966). The resistance breakpoints were defined according to criteria of the Clinical and Laboratory Standards Institute (CLSI, 2008).

Detection of class 1 integrons and Salmonella genomic island mapping

We used conserved segment polymerase chain reaction (PCR) (CS-PCR) with the 5'-CS and 3'-CS primer pair for detection of the inserted gene cassette region of class 1 integrons, as described previously (Lévesque et al., 1995). The PCR assays were performed with an initial start cycle of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2.5 min, and a final extension step of 72°C for 5 min. Amplification products were gel-purified using QIAQuick Gel Extraction kit (QIAGen, Hilden, Germany) for sequencing. The resulting DNA sequences were compared to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (www.ncbi.nlm.nih.gov).

We assayed integron-positive isolates for the presence of SGI1. The isolates were first examined by PCR for the presence of the left and right junction of SGI1. Next, the presence of sequences from the antibiotic resistance gene cluster was determined by PCR, using primers as described previously (Doublet et al., 2003; Levings et al., 2005).

RESULTS

Recovery and serotyping of Salmonella isolates

A total of 160 dogs (15.98%) tested positive for the presence of Salmonella. Of these Salmonella-positive dogs, 76 (76/483, 16%) were from shelter 1; 38 (38/301, 13%) were from shelter 2; 34 (34/125, 27%) were from shelter 3; 11 (11/52, 21%) were from shelter 4; and 1 (1/41, 2%) was from shelter 5. A total of 18.63% of female dogs and 12.72% of male dogs were positive for Salmonella. Further analysis led to the identification of 28 serotypes, including five dominant serotypes (Table 1): Salmonella Newport (n = 25, 15.63%), Salmonella Enteritidis (n = 19, 11.88%), Salmonella Senftenberg (n = 14, 8.75%), Salmonella Kalamu (n = 11, 6.88%), and Salmonella Duesseldorf (n = 10, 6.25%). The minor serotypes were Derby, Itami, Paratyphi B, Weltevreden (n = 7), Agona, Typhimurium (n = 6), Java (n = 5), Albany, Anatum, Assinie (n = 4), Braenderup (n = 3), Makumira, Panama, Schwarzengrund, Stanley (n = 2). Only one isolate was recovered for each of the following serotypes: Drypool, Kouka, Lomita, London, Reading, Saintpanl, Taksory, Tananarive. The available antisera did not allow typing of five of our S. enterica isolates.

Antimicrobial susceptibility

Among the 160 isolates, 79 (49%) were susceptible to all tested antimicrobials. The resistance rates to tested antibiotics were: tetracycline, 39% (62 of 160 isolates); streptomycin, 38% (61); nalidixic acid, 29% (47); nitrofurantoin, 28% (45); chloramphenicol, 24% (39); ampicillin, 23% (37), trimethoprim/sulphamethoxazole, 23% (37); florenicil, 20% (32), amoxycillin/clavulanic acid, 8.1% (13), and gentamicin, 1.9% (3). None of the isolates were resistant to ciprofloxacin or ceftriaxone (Table 2).

Gene cassette characterization

A total of 42 (27%) of the 160 isolates (15 different serovars) were positive for the PCR amplification product...
Table 1. *Salmonella* serotypes isolated from stray dogs in central Taiwan.

| No. of strains | S. enterica serotype* |
|----------------|-----------------------|
| 25             | Newport               |
| 19             | Enteritidis           |
| 14             | Senftenberg           |
| 11             | Kalamu                |
| 10             | Duesseldorf           |
| 7              | Derby                 |
| 7              | Itami                 |
| 7              | Paratyphi B           |
| 7              | Weltevreden           |
| 6              | Agona                 |
| 6              | Typhimurium           |
| 5              | Java                  |
| 4              | Albany                |
| 4              | Anatum                |
| 4              | Assinie               |
| 3              | Braenderup            |
| 2              | Makumira              |
| 2              | Panama                |
| 2              | Schwarzengrund        |
| 2              | Stanley               |
| 1              | Drypool               |
| 1              | Kouka                 |
| 1              | Lomita                |
| 1              | London                |
| 1              | Reading               |
| 1              | Saintpani             |
| 1              | Taksony               |
| 1              | Tananarive            |

*a*5 isolates were untypable.

Table 2. Resistance phenotypes of *Salmonella* isolates recovered from stray dogs in central Taiwan.

| Antibiotics                              | Resistant (n) | Resistant percentage |
|------------------------------------------|---------------|----------------------|
| Ampicillin                               | 37            | 23.1                 |
| Amoxicillin-clavulanic acid              | 13            | 8.1                  |
| Ceftriaxone                              | 0             | 0                    |
| Tetracycline                             | 62            | 38.8                 |
| Streptomycin                             | 61            | 38.1                 |
| Gentamicin                               | 3             | 1.9                  |
| Sulphamethoxazole/Trimethoprim           | 37            | 23.1                 |
| Chloramphenicol                          | 39            | 24.4                 |
| Florfenicol                              | 32            | 20                   |
| Nalidixic acid                           | 47            | 29.4                 |
| Ciprofloxacin                            | 0             | 0                    |
| Nitrofurantoin                           | 45            | 28.1                 |

...of class 1 integrons. Four patterns were present, 1,900, 1,200, 1,000 and 1,200 + 1,000 bp (Table 3). Sequence analysis of the integron PCR products showed the presence of classic gene cassettes in the integrons, including *aadA1* and *aadA2* (which confer resistance to streptomycin and spectinomycin), the dihydrofolate...
Table 3. Characteristics of class-1 integron-positive *Salmonella* isolates in stray dogs of central Taiwan.

| Size of amplicons (kb)* | Inserted gene cassettes | Antibiotic resistance patternb | Serotype (n)c | SGI-1 type |
|-------------------------|--------------------------|-------------------------------|--------------|------------|
| 1.0                     | *aadA*2                  | NS                            | Agona        |            |
|                         |                          | TS                            | Derby (3)    |            |
|                         |                          | AAcNSxtTCFIFS                 | Kalamu (2)   | SGI1-F     |
|                         |                          | Kalamu                        | SGI1-F       |            |
|                         |                          | AAcNSxtTCFIFS                 | Kalamu (2)   | SGI1-F     |
|                         |                          | Kalamu                        | SGI1-F       |            |
|                         |                          | AAcNSxtTCFIFS                 | Kalamu (2)   | SGI1-F     |
|                         |                          | Albany                        | SGI1-F       |            |
|                         |                          | ANSxtTCFIFS                   | Duesseldorf  | SGI1-F     |
|                         |                          | Duesseldorf (2)               | SGI1-F       |            |
|                         |                          | Kalamu                        | SGI1-F       |            |
|                         |                          | ANSxtTCFIFS                   | Albany       | SGI1-F     |
|                         |                          | Albany                        | SGI1-F       |            |
|                         |                          | ANSxtTCFIFS                   | Newport      | SGI1-F     |
|                         |                          | SxtTS                         | 3,10; i, v, -|            |
|                         |                          | AsxtTFS                       | 6,7, e, h, - |            |
| 1.2                     | *dfrA*1, *orfC*          |                               |              |            |
|                         |                          | AAcNTCFIFS                    |              |            |
|                         |                          | AAcTCFIFS                     | Te phosphurium| SGI1-A   |
|                         |                          | ANCTCFIFS                     | Derby (3)    | SGI1       |
|                         |                          | Makumira                      | SGI1         |            |
|                         |                          | NSxtTCFIFS                    | Makumira     | SGI1       |
|                         |                          | AGXTS                         | 3,10; i, v, -|            |
|                         |                          | AsxtTFS                       | 6,7, e, h, - |            |
| 1.9                     | *dfrA*12-*orfF*-*aadA*2  |                               |              |            |
|                         |                          | ANSxtTFS                      | Reading      |            |
|                         |                          | ANSxtTFGS                     | Schwarzengrund|            |
|                         |                          | ANSxtTCFGS                    | Schwarzengrund|            |
|                         |                          | AACNSxtTCFIFS                 | Stanley      |            |

*Integron profiles were defined by the number and size of the PCR amplicons, b A, ampicillin; Ac, Amoxicillin-clavulanic acid; C, chloramphenicol; Fl, flufenicol; G, gentamicin; N, nitrofurantoin; S, streptomycin; T, tetracycline; Sxt, sulphamethoxazole/trimethoprim, c Number of strains is indicated if more than one.

reductase gene cassettes *dfrA*1 and *dfrA*12 (which confer resistance to trimethoprim), and the beta-lactamase gene *bla-PSE*1 (which confers resistance to ampicillin). The integrons with the *dfrA*1-*orfC* genes were most common.

**Salmonella** genomic island 1 mapping

SGI1 was present 21 (52%) of the class 1 integron-positive isolates. SGI1 was present in three *Salmonella* Derby isolates and two *Salmonella* Makumira isolates. SGI1-A was present in a single isolate of *Salmonella* Typhimurium. SGI1-F was present in six *Salmonella* Kalamu, three *Salmonella* Duesseldorf, three

**DISCUSSION**

The prevalence of *Salmonella* spp. and of serotypes of *Salmonella* in stray dogs seem to vary among different countries (Kocabiyik et al., 2006; Tsai et al., 2007). The diet and environment of stray dogs apparently determine the number and occurrence of *Salmonella* serotypes (Finley et al., 2007; Morse and Duncan, 1975). The two most common serotypes identified in this study were also *Salmonella* Albany, one *Salmonella* Java and one *Salmonella* Newport isolates. SGI1-I was present in a single *Salmonella* Agona isolate (Table 3).
found in humans, dogs and food animals (Chen et al., 2006; Lin et al., 2009). The serotypes (Newport, Enteritidis, Derby, Paratyphi B, Weltevreden, Agona, Typhimurium, Albany, Braenderup, Schwarzengrund and Stanley) isolated from stray dogs have been implicated in human multidrug resistant Salmonella in Taiwan (Chiu et al., 2009; Lauderdale et al., 2006). We think that the Salmonella found in stray dogs may relate to human salmonellosis. Although stray animals are rarely confirmed as the source of human salmonellosis, zoonotic salmonellosis. Although stray animals are rarely found in humans, dogs and food animals (Chen et al., 2006; Lin et al., 2009). The serotypes (Newport, Enteritidis, Derby, Paratyphi B, Weltevreden, Agona, Typhimurium, Albany, Braenderup, Schwarzengrund and Stanley) isolated from stray dogs have been implicated in human multidrug resistant Salmonella in Taiwan (Chiu et al., 2009; Lauderdale et al., 2006). We think that the Salmonella found in stray dogs may relate to human salmonellosis. Although stray animals are rarely confirmed as the source of human salmonellosis, zoonotic transmission of Salmonella from sick animals may occur (Wright et al., 2005; Prescott, 2005).

Another public health concern related to Salmonella infections were the emergence and increase in antibiotic resistance among Salmonella isolates. Stray dogs rarely receive antibiotic therapy. In Taiwan, many pet dogs were abandoned by their owners. We suspect abandoned pet dogs that previously received antibiotics, this might probably be one source of multidrug resistant Salmonella. The sources of drug-resistance gene of Salmonella may also be because of antibiotics used in poultry, fishery and animal husbandry. But the kind of antibiotics allow to use in farm animals are limited. All antibiotics can be used for pets except for the drugs are poisonous to them. This means that virtually all classes of antimicrobial agents that are used for humans are also used for pets, including last-line antimicrobials.

The Salmonella isolates that we identified in this study exhibited resistance to streptomycin, ampicillin, chloramphenicol, nalidixic acid, and tetracycline. Resistance to these drugs, except for nalidixic acid and tetracycline, is associated with the presence of integrons and SGI1. The class 1 integrons examined in this study were not responsible for the total resistance phenotypes that we observed, indicating the presence of non-class 1 integron-borne resistance genes or chromosomal mutations.

The integrons detected in this study have been reported previously in a number of species, such as adaA2 in Staphylococcus aureus (GenBank accession no. AB481131) and Aeromonas punctata (Genbank accession no. FJ460178); and dfrA1/orfC in Vibrio cholerae (GenBank accession no. AB219453) and Escherichia coli (Genbank accession no. DQ018382). The dfrA12-orfF-adaA2 region of class 1 integrons was also found in Salmonella Choleraesuis isolated from human and swine (Hsu et al., 2006). The SGI1 was also found in Salmonella Typhimurium and Salmonella Derby (Chiu et al., 2006). The SGI1-A, SGI1-I or SGI1-F were not reported in Taiwan but documented in neighboring countries of Taiwan, these three kind of SGI1 were reported from human and food in China (Boyd et al., 2008), swine in Japan (Akiba et al., 2006) and fish in Thailand (Doublet et al., 2003). SGI1-F was detected from 6 different serovars in this study. This result suggested the mobility of SGI1-F better than the others SGI1. The widespread distribution of the integrons and SGI1 among numerous serotypes and across several distinct geographical regions indicated clearly that antimicrobial drug resistance genes in the integrons or SGI1 able to move from strain to strain. Transfer of plasmids with class 1 integrons or SGI1 between bacterial isolates documented (Hsu et al., 2006; Boyd et al., 2008; Leverstein-van Hall et al., 2002; Doublet et al., 2005).

In conclusion, this study documents the widespread occurrence of multidrug resistant Salmonella in stray dogs of central Taiwan and also documents the diversity of class 1 integrons and SGI1 in different Salmonella serovars. The stray dogs examined in this study may play an important role as carriers of multidrug resistant S. enterica that carry mobile genetic elements associated with resistance determinants. Human such as workers in shelters or people adopting dogs from shelters exposure to Salmonella-positive dogs has public health implications, particularly for the very young, the elderly, pregnant women, and otherwise immunocompromised individuals. The risk of transfer of resistant bacteria and resistance genes from stray dogs to humans clearly requires further investigation.

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