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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

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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 Kauffmann–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), florfenicol (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, Taksony, 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/sulfamethoxazole, 23% (37); florfenicol, 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[a]
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	Saintpanl
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)[a]	Inserted gene cassettes	Antibiotic resistance pattern[b]	Serotype (n)[c]	SGI-1 type
1.0	*aadA2*	NS	Agona	
		TS	Derby (3)	
1.2	*dfrA1*, *orfC*	AAcNSxtTCFfFS	Kalamu (2)	SGI1-F
			Kalamu	
		AAcNSxtTCFfS	Kalamu (2)	SGI1-F
			Kalamu	
		AAcNSxtTCFfS	Kalamu	SGI1-F
			Kalamu (2)	
		AAcNSxtTCFfS	Albany	SGI1-F
		ANSxtTCFfFS	Duesseldorf	SGI1-F
			Kalamu (2)	SGI1-F
			Duesseldorf (2)	
			Duesseldorf (2)	SGI1-F
		ANSxtTCFfS	Albany	
			Albany	SGI1-F
			Newport	SGI1-F
		ASxtTCFfS	Albany	SGI1-F
		NSxtTCFS	Java	SGI1-F
1.0, 1.2	*aadA2*(1.0), *bla-PSE1*(1.2)	AAcNTCFfS	Agona	
		AAcTCFfFS	Typhimurium	SGI1-A
		ANTCFfFS	Derby (3)	SGI1
			Makumira	SGI1
		ANTCFfS	Makumira	SGI1
		NSxtTCFfFS	Agona	SGI1-I
1.9	*dfrA12-orfF-aadA2*	SxtTS	Lanka	
		SxtTCS	3,10;l,v;-	
		AsxtTFS	6,7;e,h;-	
		ANSxtTFS	Reading	
		ANSxtTFGS	Schwarzengrund	
		ANSxtTCFGS	Schwarzengrund	
		AAcNSxtTCFS	Stanley	

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

reductase gene cassettes *dfrA1* and *dfrA12* (which confer resistance to trimethoprim), and the beta-lactamase gene bla_{PSE1} (which confers resistance to ampicillin). The integrons with the *dfrA1-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 *Salmonella* Albany, one *Salmonella* Java and one *Salmonella* Newport isolates. SGI1-I was present in a single *Salmonella* Agona isolate (Table 3).

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

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 (Chiou 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 *aadA2* 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-aadA2* 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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