Integron types, gene cassettes, antimicrobial resistance genes and plasmids of *Shigella sonnei* isolates from outbreaks and sporadic cases in Taiwan

Chung-Yu Chang,^{1,2} Po-Liang Lu,^{2,3,4} Chung-Che Lin,^{1,2}
Tsong-Ming Lee,⁵ Mei-Yin Tsai¹ and Lin-Li Chang^{1,2}

Correspondence

Lin-Li Chang
m725006@kmu.edu.tw

¹Department of Microbiology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC

²Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC

³Department of Internal Medicine, Kaohsiung Medical University Hospital, 100 Tzyou 1st Road, Kaohsiung 807, Taiwan, ROC

⁴Department of Laboratory Medicine, Kaohsiung Medical University Hospital, 100 Tzyou 1st Road, Kaohsiung 807, Taiwan, ROC

⁵Department of Food Science and Technology, Tajen University, 20 Weisin Road, Sin-er Village, Yanpu Township, Pingtung County 907, Taiwan, ROC

This study analysed the presence, location and transferability of integrons and antibiotic resistance genes in 103 *Shigella sonnei* outbreak isolates and in 32 sporadic isolates from Taiwan. Multiple antimicrobial resistance was common in both outbreak (95 %) and sporadic (97 %) isolates. Class 1 integrons were present in 34 outbreak isolates (33 %) and in six sporadic isolates (19 %). This study is the first, to our knowledge, to identify an atypical *sul3*-associated class 1 integron carrying the *estX-psp-aadA2-cmlA-aadA1-qacH* cassette array in *Shigella*. Class 2 integrons carrying the *dfr1-sat2-aadA1* cassette array were predominant in outbreak isolates (90 %) but were not present in sporadic isolates. Other antimicrobial resistance genes not associated with integrons were found to encode resistance to ampicillin (*bla*_{TEM}), chloramphenicol (*cat1*), sulfonamide (*sul2*) and tetracycline (*tetA* and *tetB*). The most common plasmid size was 130 kb (observed in 43 and 97 % of 1998 outbreak and sporadic isolates, respectively). In conclusion, the plasmid location of resistance genes and horizontal plasmid transfer promote the spread of multiple resistance genes in outbreak and sporadic isolates of *S. sonnei*.

Received 20 May 2010

Accepted 13 October 2010

INTRODUCTION

Shigellosis, a food- and waterborne illness caused by *Shigella* species, is now a global human health problem (Niyogi, 2005) and is a statutorily reportable communicable disease in Taiwan. *Shigella* species infections in Taiwan are commonly caused by *Shigella flexneri* and *Shigella sonnei*. *S. flexneri* has circulated mainly among aboriginal tribes in mountainous areas (Wei *et al.*, 2007). In contrast, *S. sonnei* has been implicated in most of the large shigellosis outbreaks in industrialized western Taiwan and even in imported cases (Wei *et al.*, 2007).

Appropriate antimicrobial therapy for shigellosis may reduce symptom severity and illness duration, and may also prevent potentially lethal complications and further disease transmission (Niyogi, 2007). However, the increasing resistance of *Shigella* species to widely used antimicrobials such as ampicillin, trimethoprim–sulfamethoxazole (cotrimoxazole), nalidixic acid and tetracycline is a growing concern worldwide (Ahmed *et al.*, 2006; McIver *et al.*, 2002; Oh *et al.*, 2003), as the multidrug resistance of *Shigella* usually results in treatment failure and complicates therapeutic management (Niyogi, 2007). Mobile genetic elements such as plasmids, transposons, integrons and gene cassettes reportedly enhance the acquisition and dissemination of antimicrobial resistance genes by horizontal transfer (Summers, 2006). They therefore contribute to cumulative bacterial resistance to multiple antibiotics.

The GenBank/EMBL/DDBJ accession number for the sequence of the *sul3*-associated integron in *S. sonnei* obtained in this study is FJ748514.

Multiple-antimicrobial-resistant *S. sonnei* isolates have been implicated in *S. sonnei* outbreaks in different areas of Taiwan since the 1990s (Chuang *et al.*, 2006; Huang *et al.*, 2005; Lee *et al.*, 2000, 2003). However, the molecular mechanisms of multidrug resistance in *S. sonnei* and the genetic elements involving horizontal transfer have not been investigated. The present study therefore analysed the antimicrobial resistance and related genes of *S. sonnei* isolates associated with outbreak and sporadic infections. Integrons, gene cassettes and plasmids were studied to determine their roles in disseminating antimicrobial resistance genes.

METHODS

Bacterial strains. This study analysed 103 *S. sonnei* isolates associated with several waterborne outbreaks of *S. sonnei* shigellosis in Taiwan. The isolates were isolated from outbreaks occurring in 1993 ($n=9$), 1995 ($n=20$), 1997 ($n=13$) and 1998 ($n=61$). Thirty-two *S. sonnei* isolates obtained from sporadic clinical cases in southern Taiwan clinics during 1982–1987 were also analysed. All bacterial strains were stored at -70°C in tryptic soy broth containing 15% glycerol until processing.

Antimicrobial susceptibility testing. In accordance with Clinical and Laboratory Standards Institute standards (CLSI, 2007), disc diffusion was performed to test the susceptibility of isolates to the following antimicrobials: ampicillin, aztreonam, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, imipenem, nalidixic acid, streptomycin, tetracycline and trimethoprim–sulfamethoxazole. *Escherichia coli* ATCC 25922 and ATCC 35218 were used as the reference strains for quality control of the antimicrobial agents tested. Multiple resistance was defined as resistance to three or more antimicrobials.

Integron analysis. The presence of class 1 and 2 integrons was tested by PCR using primers specific for the integron integrase genes *intI1* and *intI2* (Table 1) (Bass *et al.*, 1999; Mazel *et al.*, 2000). The DNA templates for PCR were prepared as described previously (Bass *et al.*, 1999). PCR amplification was performed in 20 μl volumes containing 5 μl bacterial template DNA, 0.2 μM each primer, 200 μM dNTP, 10 $\times$ PCR buffer and 1 U *Taq* DNA polymerase (TaKaRa) in a GeneAmp PCR System 2400 (Applied Biosystems). The PCR conditions were initial denaturation at 95°C for 1 min, 30 cycles of denaturation for 30 s at 95°C , annealing for 30 s at 65°C for *intI1* and at 58°C for *intI2*, and extension for 1 min at 72°C , and a final extension for 7 min at 72°C . Plasmid pUB2401 and plasmid ColE1::Tn7 were used as a positive control for *intI1* and *intI2*, respectively.

To characterize the gene cassettes inserted in the class 1 and 2 integrons, PCRs were performed to amplify the fragments including the cassette regions. The primers used to amplify cassettes of class 1 integrons were 5'CS and 3'CS (Table 1), as described previously (Lévesque *et al.*, 1995). The gene cassettes in *sul3*-associated class 1 integrons were amplified with two primer pairs (5'CS/cmlAR and cmlAF/sul3F; Table 1). The primers used for class 2 integrons were *intI2*AR and *tnsE* (Table 1). Gene cassettes within the integrons were then identified by nucleotide sequencing.

Detection of antimicrobial resistance genes not associated with integrons. Antimicrobial resistance genes not associated with integrons were detected by PCR. These genes included those encoding resistance to tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*) (Chen *et al.*,

2004; Randall *et al.*, 2004; Schmidt *et al.*, 2001), chloramphenicol (*cat1*, *cat2*, *cat3*, *cmlB*) (Chen *et al.*, 2004; Maynard *et al.*, 2003; Randall *et al.*, 2004), ampicillin (*bla*_{TEM}, *bla*_{SHV}) (Chen *et al.*, 2004; Maynard *et al.*, 2003) and sulfonamide (*sul2*) (Chen *et al.*, 2004). Table 1 shows the primers used to amplify these genes. Bacterial strains harbouring the known resistance genes were used as positive controls. The PCR products were confirmed by nucleotide sequencing.

Southern blot hybridization. For Southern blot hybridization, plasmid DNA was extracted using the method of Kado & Liu (1981), electrophoresed through a 0.9% agarose gel and transferred to a nylon membrane. The integrase and antimicrobial resistance genes amplified by PCR were used as probes. Non-radioactive digoxigenin labelling and detection of probes were performed according to the manufacturer's recommendations (Roche).

Conjugation experiments. Conjugation experiments were performed by liquid mating out with rifampicin-resistant *E. coli* K-12 J53-2 or nalidixic acid-resistant *E. coli* K-12 C600-1 as the recipient. Transconjugants were selected on Mueller–Hinton agar plates supplemented with appropriate antimicrobial agents as markers of antibiotic resistance encoded by donors and recipients.

RESULTS

Antimicrobial resistance of *S. sonnei*

Table 2 shows the resistance of *S. sonnei* isolates to various antimicrobial agents as revealed by antimicrobial susceptibility testing. Outbreak isolates were highly resistant to streptomycin (100%), trimethoprim–sulfamethoxazole (94%) and tetracycline (65%). Nalidixic acid resistance was noted in 23% of the 1997 isolates and in 54% of the 1998 isolates. Sporadic isolates revealed 97% resistance to chloramphenicol and tetracycline and 88% resistance to streptomycin. All isolates, whether outbreak or sporadic, were susceptible to cefotaxime, ceftriaxone, ceftazidime, aztreonam, ciprofloxacin and imipenem.

Different antimicrobial resistance patterns were found in the outbreak and sporadic *S. sonnei* isolates (Table 3). The most prevalent resistance patterns among outbreak isolates were AM-C-S-SXT-TE in 1993 (100%) and 1998 (34%) isolates, S-SXT-TE in 1995 (100%) and 1997 (77%) isolates and NA-S-SXT in 1998 (46%) isolates. The predominant resistance patterns in sporadic isolates were C-S-TE (37%) and C-NA-S-TE (25%). Overall, 98 (95%) of the 103 outbreak isolates and 31 (97%) of the 32 sporadic isolates exhibited multiple resistance.

Class 1 and class 2 integrons and gene cassettes in *S. sonnei* isolates

Table 4 shows the distribution of class 1 and class 2 integrons and their gene cassettes. Class 1 integrons were present in 34 outbreak isolates. The class 1 integrons in nine (100%) of the 1993 isolates harboured *dfr17* (encoding trimethoprim resistance) and *aadA5* (encoding streptomycin resistance) gene cassettes. Twenty-five (41%) of the 61 1998 outbreak isolates harboured class 1

Table 1. Oligonucleotides used for PCR amplification

Target	Target gene description	Primer	Nucleotide sequences (5'→3')	Amplicon size (bp)	Reference
Class 1 integrans					
<i>intI1</i>	Integrase	intI1F intI1R	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280	Bass <i>et al.</i> (1999) Bass <i>et al.</i> (1999)
Cassette region		5'CS 3'CS	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	Variable	Lévesque <i>et al.</i> (1995) Lévesque <i>et al.</i> (1995)
<i>sul1</i>	Sulfonamide resistance	sul1F sul1R	TCACCGAGGACTCCTTCTTC CAGTCCGCCTCAGCAATATC	331	Mazel <i>et al.</i> (2000) Mazel <i>et al.</i> (2000)
<i>sul3</i>	Sulfonamide resistance	sul3F sul3R	CCGATGGAGGACTTTTATTTA TAACCTAGGGCTTTGGATA	745	This study This study
<i>cmlA</i>	Chloramphenicol resistance	cmlAF cmlAR	CGCCACGGTGTTGTTGTAT GCGACCTGCGTAAATGTCAC	364	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)
Class 2 integrans					
<i>intI2</i>	Integrase	intI2C intI2A	GTAGCAAACGAGTGACGAAATG ATGTCTAACAGTCCATTTTAAATTCTA	821	Mazel <i>et al.</i> (2000) Mazel <i>et al.</i> (2000)
Cassette region		intI2AR tnsE	TAGAATTTAAAAATGGACTGTTAGACAT CCGTGTCAAAGTAGGGCATT	Variable	This study This study
Resistance genes					
<i>tetA</i>	Tetracycline resistance	TetAF TetAR	GGTTCACCTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577	Randall <i>et al.</i> (2004) Randall <i>et al.</i> (2004)
<i>tetB</i>	Tetracycline resistance	TetBF TetBR	CCTCAGCTTCTCAACGCGTG GCACCTTGCTCATGACTCTT	634	Randall <i>et al.</i> (2004) Randall <i>et al.</i> (2004)
<i>tetC</i>	Tetracycline resistance	TetCF TetCR	TCTAACAATGCGCTCATCGT GGTTGAAGGCTCTCAAGGGC	589	Schmidt <i>et al.</i> (2001) Schmidt <i>et al.</i> (2001)
<i>tetD</i>	Tetracycline resistance	TetDF TetDR	CTGGGCAGATGGTCAGATAA TGACCAGCACACCCTGTAGT	832	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)
<i>tetE</i>	Tetracycline resistance	TetEF TetER	CGTCGCCCTGTATTGTACT TGGTCAGCACCCCTTGTAAAT	814	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)
<i>tetG</i>	Tetracycline resistance	TetGF TetGR	CCGGTCTTATGGGTGCTCTA CCAGAAGAACGAAGCCAGTC	604	Randall <i>et al.</i> (2004) Randall <i>et al.</i> (2004)
<i>cmlB</i>	Chloramphenicol resistance	cmlBF cmlBR	ACTCGGCATGGACATGTACT ACGGACTGCGGAATCCATAG	840	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)
<i>cat1</i>	Chloramphenicol resistance	cat1F cat1R	CCTATAACCAGACCGTTCAG TCACAGACGGCATGATGAAC	496	Randall <i>et al.</i> (2004) Randall <i>et al.</i> (2004)
<i>cat2</i>	Chloramphenicol resistance	cat2F cat2R	ACACTTTGCCCTTTATCGTC TGAAAGCCATCACATACTGC	543	Maynard <i>et al.</i> (2003) Maynard <i>et al.</i> (2003)
<i>cat3</i>	Chloramphenicol resistance	cat3F cat3R	TTTCGCGTGAGCATTTTG TCGGATGAGTATGGGCAAC	286	Maynard <i>et al.</i> (2003) Maynard <i>et al.</i> (2003)
<i>bla_{TEM}</i>	β -Lactamase	TEM1F TEM1R	CAGCGGTAAGATCCTTGAGA ACTCCCCGTCGTGTAGATAA	643	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)
<i>bla_{SHV}</i>	β -Lactamase	SHVF SHVR	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	768	Maynard <i>et al.</i> (2003) Maynard <i>et al.</i> (2003)
<i>sul2</i>	Sulfonamide resistance	sul2F sul2R	CCTGTTTCGTCCGACACAGA GAAGCGCAGCCGAATTCAT	435	Chen <i>et al.</i> (2004) Chen <i>et al.</i> (2004)

integrans. However, the cassette regions in 24 of the 1998 isolates were not amplified by the 5'CS and 3'CS primers. These 24 isolates were found to carry an atypical class 1 integron associated with the *sul3* gene. The cassette array in the *sul3*-associated class 1 integron was *estX-psp-aadA2-cmlA1-aadA1-qacH*, which encoded an esterase/lipase, a putative phosphoserine phosphatase and resistance to

streptomycin, chloramphenicol and quaternary ammonium compounds. Six (19 %) sporadic isolates were *intI1*-positive. One isolate carried *aadA1* and five carried *dfp12-orfF-aadA2*.

Ninety-three (90 %) of the 103 outbreak isolates had class 2 integrans (Table 4). All class 2 integrans carried the gene cassette array *dfp1-sat2-aadA1*, which encoded resistance to

Table 2. Antimicrobial resistance of *S. sonnei* isolates

Results are the number of isolates (%) with antimicrobial resistance. AM, Ampicillin; C, chloramphenicol; NA, nalidixic acid; S, streptomycin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole. All isolates were susceptible to cefotaxime, ceftriaxone, ceftazidime, aztreonam, ciprofloxacin and imipenem.

Isolates	AM	C	NA	S	TE	SXT
Outbreak						
1993 (<i>n</i> =9)	9 (100)	9 (100)	0 (0)	9 (100)	9 (100)	9 (100)
1995 (<i>n</i> =20)	0 (0)	0 (0)	0 (0)	20 (100)	20 (100)	20 (100)
1997 (<i>n</i> =13)	0 (0)	0 (0)	3 (23)	13 (100)	13 (100)	13 (100)
1998 (<i>n</i> =61)	26 (43)	25 (41)	33 (54)	61 (100)	25 (41)	55 (90)
Total (<i>n</i> =103)	35 (34)	34 (33)	36 (35)	103 (100)	67 (65)	97 (94)
Sporadic (<i>n</i> =32)	6 (19)	31 (97)	18 (56)	28 (88)	31 (97)	5 (16)

trimethoprim, streptothricin and streptomycin. Class 2 integrons were more common than class 1 integrons in outbreak isolates but were not observed in sporadic isolates.

Antimicrobial resistance genes not associated with integrons

Table 4 shows the resistance genes that were not associated with integrons, including those encoding resistance to ampicillin (*bla*_{TEM}), chloramphenicol (*cat1*), sulfonamides (*sul2*) and tetracycline (*tetA* and *tetB*). The *sul2* gene was prevalent among outbreak (55 %, 57/103) and sporadic (75 %, 24/32) isolates. The *tetA* gene was common in 1995, 1997 and 1998 outbreak isolates, but the 1993 outbreak

isolates carried the *tetB* gene. The *cat1* and *tetB* genes were prevalent (97 %) in sporadic isolates.

Resistance genes carried by transferable plasmids

Plasmids of approximately 130 kb were found in 26 (43 %) 1998 outbreak isolates and in 31 (97 %) sporadic isolates and were transferable among 85 % (22/26) of outbreak isolates and among 87 % (27/31) of sporadic isolates (Table 5). In outbreak isolates, *bla*_{TEM}, *sul3* and gene cassettes associated with *sul3*-linked class 1 integrons were found within the 130 kb plasmids. In 26 of the 31 (84 %) sporadic isolates, the *cat1* and *tetB* genes were transferred along with the 130 kb plasmids. Class 2 integrons were not located on

Table 3. Antimicrobial resistance patterns of *S. sonnei* isolates

Results are the number (%) of isolates. AM, Ampicillin; C, chloramphenicol; NA, nalidixic acid; S, streptomycin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole.

Antimicrobial resistance pattern	Outbreak (<i>n</i> =103)				Sporadic (<i>n</i> =32)
	1993 (<i>n</i> =9)	1995 (<i>n</i> =20)	1997 (<i>n</i> =13)	1998 (<i>n</i> =61)	
AM-C-S-SXT-TE	9 (100)			21 (34)	1 (3)
AM-C-S-SXT				3 (5)	
AM-C-S-TE				1 (2)	
AM-S-SXT-TE				1 (2)	
NA-S-SXT-TE			3 (23)		
NA-S-SXT				28 (46)	
S-SXT-TE		20 (100)	10 (77)	2 (3)	
NA-S				5 (8)	
AM-C-NA-S-SXT-TE					3 (10)
AM-C-NA-S-TE					2 (6)
C-NA-S-TE					8 (25)
C-S-SXT-TE					1 (3)
C-NA-TE					3 (10)
C-S-TE					12 (37)
NA-S-TE					1 (3)
C-NA					1 (3)

Table 4. Integrans, gene cassette arrays and resistance genes in *S. sonnei* isolates

Isolates	Class 1 integron		Class 2 integron		No. of resistance genes without integron associations (%)				
	No. of <i>intI1</i> -positive (%)	Gene cassette array (no. of isolates)	No. of <i>intI2</i> -positive (%)	Gene cassette array (no. of isolates)	<i>bla</i> _{TEM}	<i>cat1</i>	<i>sul2</i>	<i>tetA</i>	<i>tetB</i>
Outbreak									
1993 (<i>n</i> =9)	9 (100)	<i>dfr17-aadA5</i> (9)*	0	–	9 (100)	9 (100)	–	–	9 (100)
1995 (<i>n</i> =20)	0	–	20 (100)	<i>dfr1-sat2-aadA1</i> (20)	–	–	20 (100)	20 (100)	–
1997 (<i>n</i> =13)	0	–	13 (100)	<i>dfr1-sat2-aadA1</i> (13)	–	–	13 (100)	13 (100)	–
1998 (<i>n</i> =61)	25 (41)	<i>aadA1</i> (1)*	60 (98)	<i>dfr1-sat2-aadA1</i> (60)	26 (43)	1 (2)	24 (39)	25 (41)	–
		<i>estX-psp-aadA2-cmlA1-aadA1-qacH</i> (24)†							
Sporadic (<i>n</i> =32)	6 (19)	<i>aadA1</i> (1)* <i>dfr12-orfF-aadA2</i> (5)*	0	–	5 (16)	31 (97)	24 (75)	2 (6)	31 (97)

*Gene cassette regions were amplified with primers 5'CS and 3'CS (Table 1).

†*sul3*-associated class 1 integrons.

Table 5. Plasmid-borne resistance genes and plasmid transfer in *S. sonnei* isolates

Isolates	Plasmid size in kb (no. of isolates)	Plasmid-borne resistance genes		% of plasmids (no./total)	
		Associated with class 1 integrons	Not associated with integrons	Transferable	Non-conjugative
Outbreak					
1993 (<i>n</i> =9)	126 (9)	<i>dfr17, aadA5, sul1</i>	<i>bla</i> _{TEM} , <i>cat1, tetB</i>	0	100 (9/9)
1995 (<i>n</i> =20)	8.5 (20)	–	<i>sul2, tetA</i>	0	100 (20/20)
1997 (<i>n</i> =13)	8.5 (13)	–	<i>sul2, tetA</i>	0	100 (13/13)
1998 (<i>n</i> =61)	130 (1)	<i>aadA1, sul1</i>	<i>bla</i> _{TEM} , <i>cat1, tetA</i>	0	4 (1/26)*
	130 (24)	<i>aadA2, cmlA1, aadA1, sul3</i>	<i>bla</i> _{TEM}	81 (21/26)*	11 (3/26)*
	130 (1)	–	<i>bla</i> _{TEM}	4 (1/26)*	0
	8.5 (24)	–	<i>sul2, tetA</i>	79 (19/24)	21 (5/24)
Sporadic (<i>n</i> =32)	130 (25)	–	<i>cat1, tetB</i>	78 (24/31)†	3 (1/31)†
	130 (1)	–	<i>cat1</i>	3 (1/31)†	0
	130 (1)	–	<i>tetB</i>	3 (1/31)†	0
	130 (1)	<i>aadA1, sul1</i>	<i>cat1, tetB</i>	0	3 (1/31)†
	130 (1)	<i>dfr12, aadA2, sul1</i>	<i>bla</i> _{TEM} , <i>cat1, tetA</i>	0	3 (1/31)†
	130 (1)	<i>dfr12, aadA2, sul1</i>	<i>bla</i> _{TEM} , <i>cat1, tetB</i>	3 (1/31)†	0
	130 (1)	<i>dfr12, aadA2, sul1</i>	<i>cat1</i>	0	3 (1/31)†
	140 (2)	<i>dfr12, aadA2, sul1</i>	<i>bla</i> _{TEM}	100 (2/2)	0
	148 (1)	–	<i>bla</i> _{TEM} , <i>cat1, sul2, tetB</i>	0	100 (1/1)
	6 (22)	–	<i>sul2</i>	0	100 (22/22)

*Twenty-six of the 1998 outbreak isolates harboured the 130 kb plasmid.

†Thirty-one of the sporadic isolates harboured the 130 kb plasmid.

plasmids and no class 2 integrons were transferable in conjugation experiments.

DISCUSSION

The present study revealed multiple antibiotic resistance in 95 % of outbreak isolates and in 97 % of sporadic isolates of *S. sonnei* in Taiwan. Although ampicillin and trimethoprim–sulfamethoxazole have been recommended for treating shigellosis in the past, they are currently considered ineffective for empirical therapy (Niyogi, 2005). In the current study, the observed susceptibility of *S. sonnei* isolates to ciprofloxacin, third-generation cephalosporins and imipenem indicated that these antibiotics may be the preferred empirical treatment options in Taiwan.

In Table 6, we have compared the resistance rates of the *S. sonnei* isolates with various antimicrobial agents from different time periods in Taiwan. High rates of resistance to ampicillin and trimethoprim–sulfamethoxazole were reported among outbreak *S. sonnei* isolated after 2000 (Chuang *et al.*, 2006; Huang *et al.*, 2005). Trimethoprim–sulfamethoxazole resistance was significantly higher in non-outbreak isolates from 1996 to 2005 (Wu *et al.*, 2009) than in sporadic isolates from the present study (50 vs 16 %, $P<0.05$). Wei *et al.* (2007) reported that acquisition or loss of antimicrobial resistance was noted in an *S. sonnei* clone, which evolved into many new strains with different antibiograms over a period of transmission. This suggests that antimicrobial resistance in *S. sonnei* isolates may change over time and emphasizes that continuous monitoring of resistance is necessary.

The prevalence of class 1 integrons in outbreak (33 %) and sporadic (19 %) isolates exceeded that reported in Spain (11.6 %) (Navia *et al.*, 2004), China (16.1 %) (Pan *et al.*, 2006), Japan (6 %) (Ahmed *et al.*, 2006) and Australia (7 %) (McIver *et al.*, 2002). However, a high prevalence has also been reported in Vietnam (46 %) (Iversen *et al.*, 2003). The *dfr12-orfA-aadA2*, *dfr17-aadA5* and *aadA1* cassette arrays carried by class 1 integrons in the present study have also been identified in *S. sonnei* isolates obtained in other countries, including Australia, China, Korea and Vietnam (Iversen *et al.*, 2003; McIver *et al.*, 2002; Oh *et al.*, 2003;

Pan *et al.*, 2006). These cassette arrays have been found in human and animal isolates of *E. coli* and *Salmonella* obtained from Taiwan (Chang *et al.*, 2000, 2007; Hsu *et al.*, 2006; Lee *et al.*, 2009). This indicates interspecies spread of class 1 integrons.

The present study identified an atypical class 1 integron with an unusual 3' conserved sequence linked to a *sul3* domain. This *sul3*-associated class 1 integron carried the *estX-psp-aadA2-cmlA-aadA1-qacH* cassette array, which has been identified in different bacterial species (*Salmonella* and *E. coli*), from different origins (human, animal and food), over different time periods and from different geographical areas (Antunes *et al.*, 2007; Bischoff *et al.*, 2005; Sáenz *et al.*, 2010; Vinué *et al.*, 2010). In Taiwan, *sul3*-associated class 1 integrons were recently reported in *Salmonella choleraesuis* in 2000–2001 (Lee *et al.*, 2009) and in *Klebsiella pneumoniae* in 2004 (Chang *et al.*, 2009). To our knowledge, this is the first report indicating that *Shigella* may harbour *sul3*-associated class 1 integrons. The location of this integron on transferable plasmids may enhance the spread of resistance genes within the integron.

Conversely, the observed predominance of class 2 integrons (90 % in outbreak isolates) of *S. sonnei* in this study is consistent with reports elsewhere, including studies in Australia and Korea (100 %) (McIver *et al.*, 2002; Oh *et al.*, 2003), Iran (96 %) (Ranjbar *et al.*, 2007), Senegal (93 %) (Gassama-Sow *et al.*, 2006), Japan (88 %) (Ahmed *et al.*, 2006) and China (80.6 %) (Pan *et al.*, 2006). A retrospective study by Mammina *et al.* (2006) reported that emerging class 2 integrons carrying *S. sonnei* should be backdated to the late 1980s. The current study revealed class 2 integrons in outbreak isolates obtained in 1995, 1997 and 1998, but not in outbreak isolates obtained in 1993 or in sporadic isolates obtained in 1982–1987. All class 2 integrons in this study also carried the same cassette array as that in Tn7 (*dfr1-sat2-aadA1*), which is consistent with the literature (Ahmed *et al.*, 2006; DeLappe *et al.*, 2003; McIver *et al.*, 2002; Pan *et al.*, 2006). Class 2 integrons have been found in transposon Tn7 and its relatives (DeLappe *et al.*, 2003; Gassama Sow *et al.*, 2008). Tn7 is predominantly inserted into chromosomes with high frequencies (Lichtenstein & Brenner, 1982) and can use both site-specific and non-specific transposition modes (Lichtenstein & Brenner,

Table 6. Resistance rates reported for *S. sonnei* isolated from different time periods in Taiwan

AM, Ampicillin; C, chloramphenicol; NA, nalidixic acid; S, streptomycin; TE, tetracycline; SXT, trimethoprim–sulfamethoxazole; NR, not reported.

Year	Isolates	% of resistance rate						Reference
		AM	C	NA	S	TE	SXT	
1993–1998	Outbreak ($n=103$)	34	33	35	100	65	94	This study
1999–2002	Outbreak ($n=122$)	32	NR	98	NR	NR	97.5	Chuang <i>et al.</i> (2006)
2001	Outbreak ($n=47$)	55	0	NR	NR	NR	100	Huang <i>et al.</i> (2005)
1982–1987	Sporadic ($n=32$)	19	97	56	88	97	16	This study
1996–2005	Non-outbreak ($n=12$)	8.3	NR	NR	NR	NR	50	Wu <i>et al.</i> (2009)

1982); this may contribute to dissemination of class 2 integrons along with gene cassettes located therein and may explain the predominance of class 2 integrons. A high resistance to trimethoprim and streptomycin in the outbreak *S. sonnei* isolates was associated with integrons because of the high frequency of *dfr* and *aadA* cassettes conserved within class 1 and 2 integrons, particularly in class 2 integrons. However, the high percentage (88%) of streptomycin-resistant sporadic isolates indicated that mechanisms other than integrons mediate streptomycin resistance given the low incidence of integrons observed in sporadic isolates.

The sulfonamide resistance observed in the examined *S. sonnei* isolates was conferred by *sul1*, *sul2* and *sul3* genes. The *sul1* gene, which is part of the 3' conserved segment, and the *sul3* gene, which is linked to an unusual 3' conserved sequence region, are both associated with class 1 integrons (Antunes *et al.*, 2007; Summers, 2006). The *sul2* gene is usually located on small non-conjugative plasmids or on large transmissible plasmids (Antunes *et al.*, 2005). The present study identified the *sul2* gene on small (8.5 and 6 kb) plasmids. Notably, some of the 8.5 kb plasmids were transferable.

Resistance genes, including *bla*_{TEM}, *cat1*, *sul2*, *tetA* and *tetB*, did not present as gene cassettes and were not located directly downstream of integron cassette regions. However, these resistance genes could be transferred by plasmids. In addition to the resistance genes within integrons, plasmids carrying class 1 integrons harboured other resistance genes, which promoted the co-selection of resistance upon exposure to any antimicrobial to which resistance had been encoded on plasmids.

In conclusion, this study demonstrated that multiple antibiotic resistance is common in outbreak and sporadic isolates of *S. sonnei*, especially against widely used therapeutic agents for shigellosis. Multiple antibiotic resistance in the *S. sonnei* isolates was conferred by class 1 and class 2 integrons and other antimicrobial resistance genes. Class 2 integrons, which were predominant in outbreak isolates, contributed to a high resistance to trimethoprim and streptomycin. The plasmid location of resistance genes and the horizontal transfer of plasmids facilitated the dissemination of antimicrobial resistance in outbreak and sporadic isolates of *S. sonnei*.

ACKNOWLEDGEMENTS

This study was supported by a grant from Kaohsiung Medical University (KMU92-B-01).

REFERENCES

- Ahmed, A. M., Furuta, K., Shimomura, K., Kasama, Y. & Shimamoto, T. (2006). Genetic characterization of multidrug resistance in *Shigella* spp. from Japan. *J Med Microbiol* **55**, 1685–1691.
- Antunes, P., Machado, J., Sousa, J. C. & Peixe, L. (2005). Dissemination of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*)

in Portuguese *Salmonella enterica* strains and relation with integrons. *Antimicrob Agents Chemother* **49**, 836–839.

Antunes, P., Machado, J. & Peixe, L. (2007). Dissemination of *sul3*-containing elements linked to class 1 integrons with an unusual 3' conserved sequence region among *Salmonella* isolates. *Antimicrob Agents Chemother* **51**, 1545–1548.

Bass, L., Liebert, C. A., Lee, M. D., Summers, A. O., White, D. G., Thayer, S. G. & Maurer, J. J. (1999). Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrob Agents Chemother* **43**, 2925–2929.

Bischoff, K. M., White, D. G., Hume, M. E., Poole, T. L. & Nisbet, D. J. (2005). The chloramphenicol resistance gene *cmIA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS Microbiol Lett* **243**, 285–291.

Chang, C. Y., Chang, L. L., Chang, Y. H., Lee, T. M. & Chang, S. F. (2000). Characterisation of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of *Escherichia coli* from Taiwan, ROC. *J Med Microbiol* **49**, 1097–1102.

Chang, L. L., Chang, T. M. & Chang, C. Y. (2007). Variable gene cassette patterns of class 1 integron-associated drug-resistant *Escherichia coli* in Taiwan. *Kaohsiung J Med Sci* **23**, 273–280.

Chang, C. Y., Fang, Y. T., Tsai, S. M., Chang, L. L. & Yu, W. L. (2009). Characterization of class 1 integrons and gene cassettes in clinical isolates of *Klebsiella pneumoniae* from Taiwan. *Diagn Microbiol Infect Dis* **65**, 214–216.

Chen, S., Zhao, S., White, D. G., Schroeder, C. M., Lu, R., Yang, H., McDermott, P. F., Ayers, S. & Meng, J. (2004). Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. *Appl Environ Microbiol* **70**, 1–7.

Chuang, Y. Y., Huang, Y. C. & Lin, S. Y. (2006). Outbreak of *Shigella sonnei* gastroenteritis in Northeastern Taiwan. *Pediatr Infect Dis J* **25**, 92–94.

CLSI (2007). *Performance Standards for Antimicrobial Susceptibility Testing*; 17th Informational Supplement. M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute.

DeLappe, N., O'Halloran, F., Fanning, S., Corbett-Feeney, G., Cheasty, T. & Cormican, M. (2003). Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates from western Ireland, an area of low incidence of infection. *J Clin Microbiol* **41**, 1919–1924.

Gassama-Sow, A., Diallo, M. H., Boye, C. S., Garin, B., Sire, J. M., Sow, A. I. & Aidara-Kane, A. (2006). Class 2 integron-associated antibiotic resistance in *Shigella sonnei* isolates in Dakar, Senegal. *Int J Antimicrob Agents* **27**, 267–270.

Gassama Sow, A., Diallo, M. H., Gatet, M., Denis, F., Aidara-Kane, A. & Ploy, M. C. (2008). Description of an unusual class 2 integron in *Shigella sonnei* isolates in Senegal (sub-Saharan Africa). *J Antimicrob Chemother* **62**, 843–844.

Hsu, S. C., Chiu, T. H., Pang, J. C., Hsuan-Yuan, C. H., Chang, G. N. & Tsen, H. Y. (2006). Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents* **27**, 383–391.

Huang, I. F., Chiu, C. H., Wang, M. H., Wu, C. Y., Hsieh, K. S. & Chiou, C. C. (2005). Outbreak of dysentery associated with ceftriaxone-resistant *Shigella sonnei*: first report of plasmid-mediated CMY-2-type AmpC β -lactamase resistance in *S. sonnei*. *J Clin Microbiol* **43**, 2608–2612.

Iversen, J., Sandvang, D., Srijan, A., Cam, P. D. & Dalsgaard, A. (2003). Characterization of antimicrobial resistance, plasmids, and gene cassettes in *Shigella* spp. from patients in Vietnam. *Microb Drug Resist* **9**, S17–S24.

- Kado, C. I. & Liu, S. T. (1981). Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* **145**, 1365–1373.
- Lee, T. M., Chang, L. L., Chang, C. Y., Wang, J. C., Pan, T. M., Wang, T. K. & Chang, S. F. (2000). Molecular analysis of *Shigella sonnei* isolated from three well-documented outbreaks in school children. *J Med Microbiol* **49**, 355–360.
- Lee, T. M., Chang, C. Y., Chang, L. L., Chen, W. M., Wang, T. K. & Chang, S. F. (2003). One predominant type of genetically closely related *Shigella sonnei* prevalent in four sequential outbreaks in school children. *Diagn Microbiol Infect Dis* **45**, 173–181.
- Lee, M. F., Chen, Y. H. & Peng, C. F. (2009). Molecular characterisation of class 1 integrons in *Salmonella enterica* serovar Choleraesuis isolates from southern Taiwan. *Int J Antimicrob Agents* **33**, 216–222.
- Lévesque, C., Piché, L., Larose, C. & Roy, P. H. (1995). PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* **39**, 185–191.
- Lichtenstein, C. & Brenner, S. (1982). Unique insertion site of Tn7 in the *E. coli* chromosome. *Nature* **297**, 601–603.
- Mammina, C., Aleo, A., Romani, C. & Nastasi, A. (2006). *Shigella sonnei* biotype g carrying class 2 integrons in southern Italy: a retrospective typing study by pulsed field gel electrophoresis. *BMC Infect Dis* **6**, 117.
- Maynard, C., Fairbrother, J. M., Bekal, S., Sanschagrin, F., Lévesque, R. C., Brousseau, R., Masson, L., Larivière, S. & Harel, J. (2003). Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. *Antimicrob Agents Chemother* **47**, 3214–3221.
- Mazel, D., Dychinco, B., Webb, V. A. & Davies, J. (2000). Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. *Antimicrob Agents Chemother* **44**, 1568–1574.
- McIver, C. J., White, P. A., Jones, L. A., Karagiannis, T., Harkness, J., Marriott, D. & Rawlinson, W. D. (2002). Epidemic strains of *Shigella sonnei* biotype g carrying integrons. *J Clin Microbiol* **40**, 1538–1540.
- Navia, M. M., Ruiz, J. & Vila, J. (2004). Molecular characterization of the integrons in *Shigella* strains isolated from patients with traveler's diarrhea. *Diagn Microbiol Infect Dis* **48**, 175–179.
- Niyogi, S. K. (2005). Shigellosis. *J Microbiol* **43**, 133–143.
- Niyogi, S. K. (2007). Increasing antimicrobial resistance – an emerging problem in the treatment of shigellosis. *Clin Microbiol Infect* **13**, 1141–1143.
- Oh, J. Y., Yu, H. S., Kim, S. K., Seol, S. Y., Cho, D. T. & Lee, J. C. (2003). Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from southwestern Korea during epidemic periods. *J Clin Microbiol* **41**, 421–423.
- Pan, J. C., Ye, R., Meng, D. M., Zhang, W., Wang, H. Q. & Liu, K. Z. (2006). Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. *J Antimicrob Chemother* **58**, 288–296.
- Randall, L. P., Cooles, S. W., Osborn, M. K., Piddock, L. J. & Woodward, M. J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *J Antimicrob Chemother* **53**, 208–216.
- Ranjbar, R., Aleo, A., Giammanco, G. M., Dionisi, A. M., Sadeghifard, N. & Mammina, C. (2007). Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002–2003. *BMC Infect Dis* **7**, 62.
- Sáenz, Y., Vinué, L., Ruiz, E., Somalo, S., Martínez, S., Rojo-Bezares, B., Zarazaga, M. & Torres, C. (2010). Class 1 integrons lacking *qacEΔ1* and *sul1* genes in *Escherichia coli* isolates of food, animal and human origins. *Vet Microbiol* **144**, 493–497.
- Schmidt, A. S., Bruun, M. S., Dalsgaard, I. & Larsen, J. L. (2001). Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. *Appl Environ Microbiol* **67**, 5675–5682.
- Summers, A. O. (2006). Genetic linkage and horizontal gene transfer, the roots of the antibiotic multi-resistance problem. *Anim Biotechnol* **17**, 125–135.
- Vinué, L., Sáenz, Y., Rojo-Bezares, B., Olarte, I., Undabeitia, E., Somalo, S., Zarazaga, M. & Torres, C. (2010). Genetic environment of *sul* genes and characterisation of integrons in *Escherichia coli* isolates of blood origin in a Spanish hospital. *Int J Antimicrob Agents* **35**, 492–496.
- Wei, H. L., Wang, Y. W., Li, C. C., Tung, S. K. & Chiou, C. S. (2007). Epidemiology and evolution of genotype and antimicrobial resistance of an imported *Shigella sonnei* clone circulating in central Taiwan. *Diagn Microbiol Infect Dis* **58**, 469–475.
- Wu, C. H., Huang, L. T., Huang, I. F., Liu, J. W., Chen, J. B., Liang, C. D., Hwang, K. P. & Tiao, M. M. (2009). Acute non-outbreak shigellosis: ten years experience in southern Taiwan. *Chang Gung Med J* **32**, 59–65.