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Epidemiological Typing and Characterization of *dfr* Genes of *Shigella sonnei* Isolates in Korea During the Last Two Decades

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Abstract One-hundred and twenty-four trimethoprim-resistant *Shigella sonnei* isolates extracted in Korea during the last two decades were investigated for their epidemiological relationship and mechanisms of resistance to trimethoprim. The *S. sonnei* isolates were distributed into two groups by three different epidemiological tools: biotyping, antibiogram, and pulsed-field gel electrophoresis. One group contained the isolates from the 1980s and the other group included the isolates from the 1990s. The geometric mean MICs of trimethoprim in *S. sonnei* isolates from the 1980s and 1990s were found to be 672.9 μg/ml and >2,048 μg/ml, respectively. Trimethoprim resistance was associated with *dfrA5*, *dfrA12*, and *dfrA13* genes in the isolates from the 1980s, *dfrA1*, *dfrA5*, and *dfrA12* in the isolates from 1991, and *dfrA1* and *dfrA12* in the isolates from 1992 to 1999. The *dfrA1* gene was located downstream of the *intI2* gene in Tn7, which was located on chromosome. Some *dfrA12* genes were found as gene cassettes in the class 1 integron. The *dfrA5* and *dfrA13* genes were located on conjugative plasmids. These results suggested that a clonal change occurred in *S. sonnei* isolates in Korea during the last two decades and that *dfr* genes located on different transposable genetic elements had gradually changed.

Key words: *Shigella sonnei*, epidemiology, trimethoprim, *dfr* gene

Shigellosis is an important cause of acute diarrheal disease in both developing and industrialized countries. *Shigella sonnei* is the predominant species in industrialized countries [32, 44]. The isolation rates of *S. sonnei* species were very low in Korea before 1990 and represented less than 15% of shigellosis, but large outbreaks of *S. sonnei* infection were reported in different parts of Korea during the period of 1998 to 2000 [29, 30]. Thus, *S. sonnei* infection is a serious public health problem in Korea. The explosive increases of *S. sonnei* infections in Korea raised a question of whether these were due to spread of an epidemic clone or increased size of sporadic infections. To analyze the epidemiological relationship of *Shigella* isolates, several conventional and molecular typing methods have been introduced [24-26]. Antibiogram, biotyping, plasmid profile, ribotyping, and pulsed-field gel electrophoresis (PFGE) were used to discriminate *Shigella* strains. On the other hand, the understanding of antimicrobial susceptibilities in multiresistant *S. sonnei* strains may contribute to the creation of new antimicrobial strategies. Furthermore the study of the mechanisms of antimicrobial resistance may provide insight into the means by which the resistant gene is spreading among the bacterial population.

Trimethoprim is a broad spectrum antimicrobial agent used against enteric pathogens such as *E. coli* and *Shigella* species. However, trimethoprim resistance among bacterial pathogens has increased worldwide during the last two decades [7, 13, 19]. A variety of resistance mechanisms to trimethoprim have been identified in clinically important bacteria. The most commonly encountered mechanism is the acquisition of a foreign *dfr* gene coding for an additional dihydrofolate reductase enzyme, which is less sensitive to the inhibition of trimethoprim [2]. More than 16 of these variant enzymes have been identified in Gram-negative bacteria [19]. The most commonly found *dfr* gene in Gram-negative bacteria is *dfrA1*, which confers high level of resistance to trimethoprim [16, 18, 43]. The location of *dfrA1* on a transposon 7 (Tn7) may explain the wide disseminations of trimethoprim resistance [4, 14]. Moreover, trimethoprim-resistant *dfr* genes have mainly been found as gene cassettes in integrons [12, 33, 43]. When *dfr* genes become a part of a gene cassette, they acquire the ability to move readily from one genome to another. The horizontal spread of transposons and integrons carrying the *dfr* gene

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is responsible for the disseminations of trimethoprim resistance.

In the previous study, high frequencies of trimethoprim-resistant *S. sonnei* isolates in Korea were found [21]. *S. sonnei* isolates from the 1990s were highly resistant to trimethoprim (MIC of ≥2,048 μg/ml), while MICs to trimethoprim of *S. sonnei* isolated in the 1980s were much lower (geometric mean MIC of 604.3 μg/ml) than those isolated in the 1990s. In this study, the epidemiological relationship and the distribution of *dfr* genes in trimethoprim-resistant *S. sonnei* isolates in Korea during the last two decades were investigated. Furthermore, the genetic organization of *dfr* genes with transposable genetic elements was characterized.

MATERIALS AND METHODS

Bacterial Strains

A total of 128 *S. sonnei* species were isolated in Korea during the period of 1980 to 1999. Thirty-nine strains were isolated from the Kyungpook province during the period of 1980 to 1986. Twelve strains from 1991 to 1997 were received from the different diagnostic laboratories in Korea. During this period, the frequencies of shigellosis were less than 100 cases per year [30]. Seventy-seven strains were isolated from two areas, Kyungpook and Chunnam provinces, during the period of 1998 to 1999. Of the 77 strains, 10 were isolated from a defined outbreak. The 124 isolates were selected according to their resistance to trimethoprim (MIC ≥16 μg/ml). *S. sonnei* species were identified by biochemical tests and serotyping with specific antisera (Difco Laboratories, Detroit, MI, U.S.A.) [6].

Biochemical Typing

Biotype was determined with the API20E kit (bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions. The fermentation of xylose was determined on phenol red broth base (Difco Laboratories) supplemented with 1% of xylose. *S. sonnei* isolates were inoculated on the media and incubated at 37°C for 20 h. Biotypes were classified by the method of Nastasi *et al.* [27].

Antimicrobial Susceptibility Testing

The MICs of the antimicrobial agents were determined by agar dilution in Mueller-Hinton agar medium (Difco Laboratories) with a Steers multiple inoculator [38] according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [28]. The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited the growth of the organism [22]. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The antimicrobial agents included were chloramphenicol, nalidixic acid, sulfamethoxazole, tetracycline, trimethoprim (all of the antimicrobial agents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.), kanamycin (Dong A Pharma Co., Seoul, Korea), and ampicillin (Young Jin Pharmatheutical Co., Seoul, Korea).

Transfer of Trimethoprim Resistance Determinants

E. coli RG488 Rif^r and *E. coli* J53 Azi^r were used as recipients for conjugation experiments. Donor and recipient strains at logarithmic phase were grown in trypticase soy broth (TSB, Difco Laboratories), and were mixed with equal volumes and the mixture was incubated at 37°C for 20 h. Transconjugants were selected on Mueller-Hinton agar medium supplemented with trimethoprim (50 μg/ml) and rifampin (50 μg/ml) or sodium azide (50 μg/ml).

Bacterial DNA Isolation

The organisms were inoculated into 4 ml of TSB and incubated at 37°C for 20 h with shaking. Cells were harvested by centrifugation at 12,000 rpm for 10 min. After the supernatant was removed, the pellet was suspended in TE buffer (25 mM Tris, pH 8.0, 10 mM EDTA) supplemented with 50 mM dextrose. Plasmid DNA was isolated by the alkaline extraction method [5], and genomic DNA was isolated as described previously [3]. The extracted plasmids and genomic DNAs were separated by electrophoresis on 0.7% agarose gel.

PFGE

Genomic DNA was digested with *Xba*I (Boehringer Mannheim, Mannheim, Germany) for 20 h and separated on 1% agarose gel using a contour-clamped homogeneous-field apparatus (CHEF-DR2, Bio-Rad Laboratories, Hercules, CA, U.S.A.) [17]. The conditions for electrophoresis were 6 V/cm for 21 h, with pulse times increasing from 5 to 40 sec. PFGE patterns were interpreted using the criteria established by Tenover *et al.* [41].

PCR Amplification of *dfr* Genes

The PCR was performed in a total volume of 20 μl containing the following: 2 μl of boiled bacterial suspensions, 50 pM of each primer, 250 μM dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1.5 U of *Taq* DNA polymerase (TaKaRa Shuzo Co. Ltd, Shiga, Japan). The template was prepared by suspending a loopful of each isolate, which had been growing on a trypticase soy agar (Difco Laboratories) plate, in 200 μl of sterile water, followed by boiling for 10 min and centrifugation for 5 min. The primers D1 (5'-ACGGATCCTGGCTGTTGG-TTGGACGC-3') and D2 (5'-CGGAATTCACCTTCCGG-CTCGATGTC-3'), described previously by Gibreel and Sköld [10], were used for the detection of the *dfrA1* gene. The primer set of D3 (5'-GTTGCGGTCCAGACATAC-3') and D4 (5'-CCGCCACCAGACACTA-3') was designed to co-amplify the *dfrA5* and *dfrA14* genes [20]. The primers

D7 (5'-CCGTGGGTCGATGTTTGATG-3') and D8 (5'-GCATTGGGAAGAAGGCGTCAC-3') co-amplified the *dfrA12* and *dfrA13* genes [20]. The PCR products of the *dfrA13* and *dfrA14* genes possessed a restriction site for *Eco*RV and *Eco*RI, respectively. The amplification reaction consisted of 30 cycles of denaturation at 94°C for 30 sec, various annealing temperatures (58°C for the primers D1 and D2, and 54°C for the primers D7 and D8) for 30 sec, and extension at 72°C for 1 min. The PCR was completed with a final elongation step of 5 min at 72°C.

PCR Amplification of Integrons

To amplify class 1 integrons and the *intI2* gene of Tn7, primer pairs 5'CS (5'-GGCATCCAAGCAGCAAG-3') and 3'CS (5'-AAGCAGACTTGACCTGA-3'), and IntI2A and IntI2B were used, respectively [39, 42]. The primers IntI2A (5'-ATGTCTAACAGTCCATTTTTAAATTCTA-3') and IntI2B (5'-AAATCTTTAACCCGCAAACGC-3') are located within the *intI2* gene of Tn7. The PCR was performed as described previously [20].

Characterization of the Genetic Organization of *dfrA1* Gene

To determine the occurrence of the *dfrA1* gene in connection with the *intI2* gene of Tn7, primers Tn7int and DHFR1C were used. The Tn7int (5'-ATTGGATCCTGATTGATAAGTAG-3') is located within the *intI2* gene of Tn7 and the DHFR1C (5'-GGCACTCCATGGAATATCAG-3') is present at the start of the *dfrA1* gene [9, 36]. The size of the expected amplification products was approximately 1.2 kb in the case of Tn7. The amplification reaction was 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. The cycles were then terminated by final elongation for 10 min at 72°C.

Southern Hybridization

After agarose gel electrophoresis of DNAs, denatured DNAs were transferred onto positively charged nylon membrane (Hybond-N+; Amersham, Braunschweig, Germany) by the capillary methods [34]. For hybridization assays, DIG DNA labeling and detection kits (Boehringer Mannheim) were used according to the manufacturer's instructions. The hybridization was performed under high-stringency conditions [35]. The probes were labeled with digoxigenin-11-dUTP by random labeling methods.

Cloning and Sequencing

The PCR products of class 1 integrons were ligated with pGEM T-easy vector (Promega, Madison, WI, U.S.A.) and were used to transform *E. coli* DH5α cells. Sequencing reactions were performed with double-stranded plasmid preparations by dideoxy chain termination with universal primer T7.

RESULTS

Of the 128 isolates tested, 124 (96.9%) isolates were resistant to trimethoprim. The MICs of trimethoprim in 35 isolates from the 1980s ranged from 256 to ≥2,048 µg/ml, while the MICs in 89 isolates from the 1990s were ≥2,048 µg/ml. The 124 trimethoprim-resistant *S. sonnei* isolates were further investigated.

Epidemiological Typing of Trimethoprim-Resistant *S. sonnei* Isolates

To determine the clonal relationship of *S. sonnei* isolates, three different epidemiological tools (biotyping, antibiogram, and PFGE) were used. The isolates showed two different

Table 1. Characterization of *S. sonnei* isolates by three different epidemiological markers.

Group (no)	Biotype			Antibiogram*	PFGE	Isolated year
	ONPG	Rhamnose	Xylose			
A (30)	+	+	–	CmTcSuTp	Ia	1980–86
(2)	+	+	–	CmTcSuTp	Ib	1982; 84
(1)	+	+	–	CmTcSuTpAp	Ib	1984
(1)	+	+	–	CmTcSuTpNa	Ib	1985
(1)	+	+	–	CmTcSuTpAp	IIa	1983
B (6)	+	–	–	TcSuTp	IIb	1992–97
(5)	+	–	–	TcSuTpApKm	IIc	1991
(1)	+	–	–	SuTpApKm	IIc	1991
(61)	+	–	–	TcSuTpNa	IIc	1998; 99
(12)	+	–	–	TcSuTpNaAp	IIc	1998; 99
(2)	+	–	–	TpNa	IIc	1998; 99
(1)	+	–	–	TcTpNa	IIc	1999
(1)	+	–	–	TcSuTpNaKm	IId	1999

*Abbreviations: Cm, chloramphenicol; Tc, tetracycline; Su, sulfamethoxazole; Tp, trimethoprim; Ap, ampicillin; Na, nalidixic acids; Km, kanamycin.

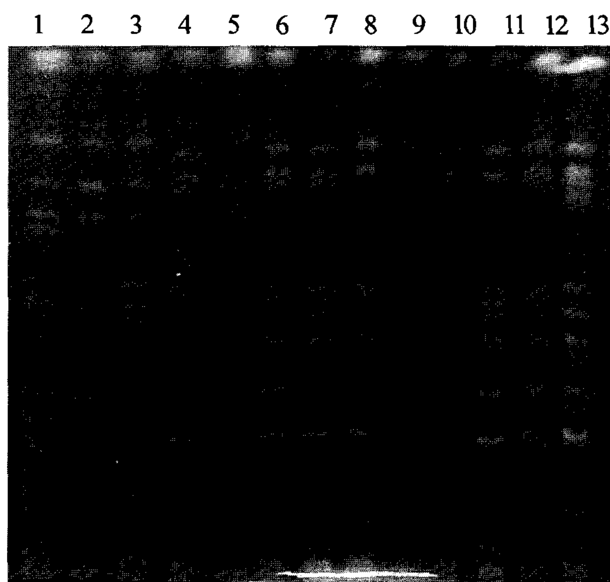

Fig. 1. PFGE of the *XbaI*-cleaved genomic DNAs of *S. sonnei* isolates.
Lane 1, Ia; lanes 2, 3, and 5, Ib; lane 4, IIa; lane 6, IIb; lanes 7, 8, 9, 10, 11, and 12, IIc; lane 13, IId.

biotypes of rhamnose by fermentation: The isolates from the 1980s fermentated rhamnose, while the isolates from the 1990s did not (Table 1). According to the antimicrobial susceptibility test, 11 antibiograms were defined. The isolates from the 1980s were resistant to chloramphenicol, while the isolates from 1998 and 1999 were resistant to nalidixic acid. *S. sonnei* isolates were distributed into two groups by PFGE, arbitrarily designated as patterns I and II (Fig. 1). Two subgroups of PFGE pattern I differed by two bands, which indicated they were epidemiologically closely related. PFGE pattern Ia included 30 isolates among the 35 isolates from the 1980s. PFGE pattern II included all the isolates from the 1990s and one isolate from 1983. PFGE pattern II was subgrouped into four parts, which differed by four bands. PFGE pattern IIc was the most common type. Eighty-two isolates belonging to PFGE pattern IIc were distributed in six groups, based on the antibiogram (Table 1).

Identification of the Trimethoprim-Resistant *dfr* Genes

PCR was performed to detect the trimethoprim-resistant *dfr* genes (Fig. 2). Of the 35 isolates of *S. sonnei* examined during the period of 1980 to 1986, 31 carried the *dfrA5* gene, 2 carried the *dfrA5* and *dfrA12* genes, and 1 carried the *dfrA13* gene (Table 2). One isolate showed a negative result when tested for the *dfrA1*, *dfrA5*, *dfrA12*, *dfrA13*, and *dfrA14* genes. Three different *dfr* genes (*dfrA1*, *dfrA5*, and *dfrA12*) were amplified in the six isolates from 1991. All 83 *S. sonnei* isolates examined between 1992 and 1999 carried the *dfrA1* and *dfrA12* genes.

Transfer of Trimethoprim Resistance Determinants

Trimethoprim resistance was transferred to a recipient *E. coli* in all *S. sonnei* isolates from the 1980s and 1991, while *S. sonnei* isolates from 1992 to 1999 could not conjugally transfer their antimicrobial resistance (Table 2). PCR amplification of transconjugants and subsequent Southern hybridization indicated that the *dfrA5* and *dfrA13* genes were located on conjugative plasmids. The *dfrA12* gene of *S. sonnei* isolated in the 1980s and 1991 was located on conjugative plasmids, while that of *S. sonnei* isolated from 1992 to 1999 was located on nonconjugative plasmids.

Association of *dfr* Genes with Integrons

To determine the association of the *dfr* gene with mobile genetic elements, we first analyzed the presence of class 1 integrons by PCR with the primers 5'CS and 3'CS. Of the 124 *S. sonnei* isolates, PCR products of 1,009 bp and 1,911 bp were amplified in 33 and 18 isolates, respectively (Fig. 3). All the isolates carrying the class 1 integrons of 1,009 bp were isolated from the 1980s. Among the 18 *S. sonnei* isolates carrying the class 1 integrons of 1,911 bp, 2, 6, and 10 strains were isolated from the 1980s, 1991, and 1999, respectively. Ten strains that originated from a defined outbreak showed positive for the class 1 integron of 1,911 bp. To investigate the presence of the *dfr* gene as a gene cassette in class 1 integrons, each of the 1,009 bp and 1,911 bp fragments were extracted from agarose gel and cloned into the vector for nucleotide sequencing. The nucleotide sequence of 1,009 bp showed the presence of an *aadA* gene cassette. Integrons of 1,911 bp carried the three gene cassettes, *dfrA12*, *orfF*, and *aadA2*.

Genetic Organization of *dfrA1* Gene

In order to determine the genetic location of the *dfrA1* gene, Southern hybridization of extracted genomic or plasmid

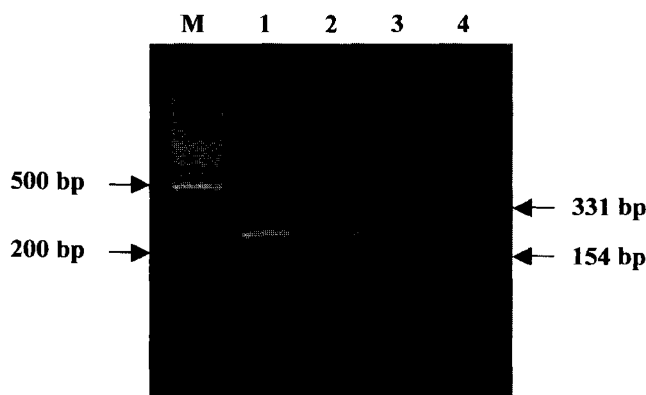

Fig. 2. PCR amplification of the trimethoprim-resistant *dfr* genes.
Lane 1, *dfrA1* gene (257 bp) with the primers D1 and D2; lane 2, *dfrA5* gene (253 bp) with the primers D3 and D4; lane 3, *dfrA12* gene (485 bp) with the primers D7 and D8. The PCR products of the *dfrA13* gene with the primers D7 and D8 possessed a restriction site for *Eco*RV (lane 4). Lane M, 100 bp DNA ladder.

Table 2. The *dfr* genes and presence of integrons in *S. sonnei* isolates.

Isolated year/ Antimicrobial resistance[a]	No. of isolates	No. of strains carrying				Integrons	
		dfrA1	*dfrA5*	*dfrA12*	*dfrA13*	Class 1	Tn7
1980–1986							
CmTcSuTp	31	0	30 (30)[c]	0	1 (1)	+ (1,009 bp)	-
CmTcSuTp	1	0	1 (1)	1 (1)	0	+ (1,911 bp)	-
CmTcSuTpAp	2[b]	0	1 (1)	1 (1)	0	+ (1,911 bp)	-
CmTcSuTpNa	1	0	1 (1)	0	0	+ (1,009 bp)	-
1991							
TcSuTpApKm	5	5 (0)	5 (5)	5 (5)	0	+ (1,911 bp)	+[d]
SuTpApKm	1	1 (0)	1 (1)	1 (1)	0	+ (1,911 bp)	+
1992-1997							
TcSuTp	6	6 (0)	0	6 (0)	0	-	+
1998–1999							
TcSuTpNa	61	0	61 (0)	0		-	+
TcSuTpNaAp	12	12 (0)	0	12 (0)	0	+ (1,911 bp)[e]	+
TpNa	2	2 (0)	0	2 (0)	0	-	+
TcTpNa	1	1 (0)	0	1 (0)	0	-	+
TcSuTpNaKm	1	1 (0)	0	1 (0)	0	-	+

[a]Abbreviations: Cm, chloramphenicol; Tc, tetracycline; Su, sulfamethoxazole; Tp, trimethoprim; Ap, ampicillin; Na, nalidixic acids; Km, kanamycin.
[b]Of the two isolates, one carried the unknown *dfr* gene and class 1 integron of 1,009 bp.
[c]The numbers in parenthesis were transconjugants that were transferred the trimethoprim-resistant *dfr* gene.
[d]The *intI2* gene of class 2 integrons was amplified with the primers IntI2A and IntI2B.
[e]Ten strains, which were originated from a defined outbreak, carried the class 1 integron of 1,911 bp.

DNAs was performed with a *dfrA1* gene probe. The *dfrA1* gene was detected on chromosomes in all of the 89 isolates. The internal fragments of the *intI2* gene were amplified using IntI2A and IntI2B primers to determine the occurrence of Tn7. All *S. sonnei* isolates carrying the *dfrA1* gene exhibited positive PCR results with the length of 440 bp, indicating the occurrence of the *intI2* gene specific for Tn7. To investigate the genetic organization of the *dfrA1* gene on the chromosomes of *S. sonnei*, PCR amplification with the primers DHFR1C and Tn7int, which detect the contribution of Tn7 to the *dfrA1* gene, was performed. Of the 89 isolates tested, all the isolates were found to carry the *dfrA1* gene downstream of the *intI2* gene specific for Tn7.

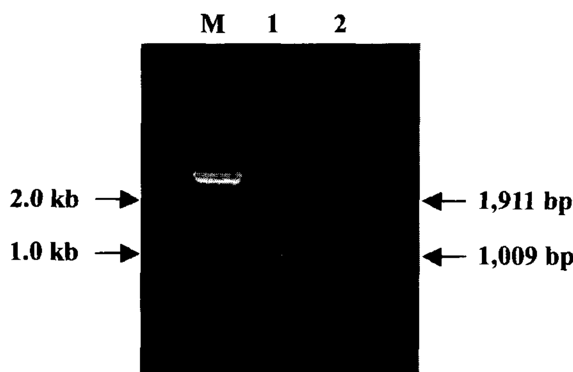

Fig. 3. PCR amplification of class 1 integrons by using primers 5'CS and 3'CS.
DNA templates were from *S. sonnei* 80DH168 (lane 1) and 83DH128 (lane 2). Lane M, 1.0 kb DNA ladder.

DISCUSSION

S. sonnei isolates in Korea during the last two decades showed high level of resistance to trimethoprim, which was invariably associated with resistance to multiple antimicrobial agents. The isolates from the 1990s had higher level of resistance to trimethoprim than the sporadic isolates from the 1980s. To determine whether the differences of MICs of trimethoprim between the isolates from the 1980s and 1990s were due to clonal changes or the presence of the different *dfr* genes of identical clones, *S. sonnei* isolates were investigated for their epidemiological relationship. Trimethoprim-resistant *S. sonnei* isolates were distributed into two distinct groups by biotyping and PFGE. One group contained the isolates from the 1980s and the other group included the isolates from the 1990s (Table 1). Antibiograms also showed two different patterns of resistance to either chloramphenicol or nalidixic acid. Taking PFGE as a reference epidemiological tool, strains belonging to the same PFGE group but having different antibiograms were observed. This was probably due to the presence or absence of plasmids, which carried antimicrobial-resistant

genes. Although one isolate (83DH128) belonged to PFGE pattern IIa, this isolate showed a different biotype and antibiogram with the isolates from the 1990s. Epidemiological study, therefore, indicated that a clonal change occurred in *S. sonnei* in Korea during the last two decades.

It was demonstrated how the prevalence of different trimethoprim-resistant *dfr* genes changed with time in *S. sonnei* isolates during the last two decades. Trimethoprim resistance was mediated by *dfrA5*, *dfrA12*, and *dfrA13* genes in the isolates from the 1980s, *dfrA1*, *dfrA5*, and *dfrA12* in the isolates from 1991, and *dfrA1* and *dfrA12* in the isolates from 1992 to 1999 (Table 2). *S. sonnei* isolates used in this study showed a negative result when tested for the presence of *dfrA7*, *dfrA8*, *dfrA14*, and *dfrA17* (data not shown). The majority of isolates (94.3%) from the 1980s carried the *dfrA5* gene, while all the isolates from the 1990s carried the *dfrA1* and *dfrA12* genes. The different *dfr* genes explained the differences of MICs to trimethoprim in the *S. sonnei* isolates. It has been known that the *dfrA5* gene was originally observed in isolates from Sri Lanka in the early 1980s and had spread to geographically restricted regions [1, 11, 13, 40]. During the 1980s, the *dfrA5* gene was also widely disseminated in *S. sonnei* and was responsible for 94.3% of observed trimethoprim resistance.

Integrons have been known as vehicles for the transmission of antimicrobial resistance genes [12, 33]. Recently, many *dfr* genes have been found as gene cassettes inserted in the active sites of integrons [15, 39, 43]. The transmission of integrons borne by a *dfr* gene to bacteria results in the acquisition of a foreign *dfr* gene expressing trimethoprim resistance. On the basis of antimicrobial susceptibility and subsequent conjugation experiment, the presence of sulfonamide resistance is strongly suggestive of the presence of class 1 integrons when it is carried by conjugative plasmids [33]. In this study, we found two different class 1 integrons in *S. sonnei* isolates. The sequences of 1,911 bp, which carried the *dfrA12*, *orfF*, and *aadA2* genes, were identical to those in *K. pneumoniae* KpS15 (GenBank accession no. AF180731) from 32 to 1,942 bases. *K. pneumoniae* KpS15 strain was first isolated in Korea. Class 1 integrons of 1,911 bp are located on plasmids, confirming that integrons may be transferred via plasmids between these two bacterial species. The integron of 1,009 bp, which carried the *aadA* gene, was identical to that reported by Clark *et al.* [8] for *E. faecalis* W4770 (GenBank accession no. AF052459). The integrons borne by the *dfr* gene cassette found in *Shigella* species have previously been identified. The *dfrA7* gene was found within the class 1 integrons of *S. flexneri* strains isolated in Tanzania [31], and the *dfrA12* gene cassette in *Shigella* strains was described in Finland [15].

The prevalence of nontransferable resistance to a high level of trimethoprim and to streptomycin suggested that Tn7 had been transposed to bacterial chromosomes [23, 37]. *S. sonnei* isolates from the 1990s carried the *dfrA1* gene located on Tn7, while the *dfrA1* gene was not found in the isolates from the 1980s. The *dfrA1* gene located on Tn7 was detected by PCR with the specific primers to amplify a part of the *dfrA1* gene and the *intI2* gene of Tn7. All the isolates carrying the *dfrA1* gene showed resistance to very high level of trimethoprim (MIC of ≥2,048 μg/ml). Six *S. sonnei* isolates from 1991 carried the three *dfr* genes, *dfrA1*, *dfrA5*, and *dfrA12*. We observed three different genetic locations of *dfr* genes in these isolates. The *dfrA1* gene occurred as a part of Tn7, which was located on chromosome, and the *dfrA5* gene was located on conjugative plasmids. The *dfrA12* gene occurred as a gene cassette in class 1 integrons, which was located on conjugative plasmids. This indicates that *dfr* genes have been horizontally spread via plasmids or transposons.

The present findings showed that two different clones of trimethoprim-resistant *S. sonnei* were spread in Korea and the *dfr* genes have gradually changed during the last two decades. *S. sonnei* maintained the resistance to trimethoprim by the mediation of transposable genetic elements carrying *dfr* genes, although trimethoprim in combination with sulfamethoxazole has no longer been used for the treatment of shigellosis.

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